VOL. 31 AUGUST, 1946 No. 8

The Journal of Laboratory and Clinical Medicine

Clinical and Experimental

The Elimination of Human Reaction-Producing Material From Horse-Derived Antiserums: An Electrical Method. Eugene Cardone, New York, N. Y. ---------- 829

A Study of Abnormal T Waves in Patients Presenting No Evidence of Organic Heart Disease. Isidore Stein, M.D., Brooklyn, N. Y. ---------- 837

Observations on the Effect of Theophylline Aminoisobutanol in Experimental Heart Failure. John R. Smith, M.D., and Julius Jensen, M.D., St. Louis, Mo. ------ 850

The Effect of Theophylline Aminoisobutanol on the Circulation in Congestive Heart Failure. Franz U. Steinberg, M.D., and Julius Jensen, M.D., St. Louis, Mo. 857

Stimulation of the Central Nervous System by Curare (Intocostrin). Rosellen E. Cohnberg, St. Louis, Mo. ---------- 866

Hemoglobin Levels in Specific Race, Age, and Sex Groups of a Normal North Carolina Population. D. F. Milam and Hugo Muench, New York, N. Y. ---- 878

Hematologic Survey of Repatriated American Military Personnel. Lieutenant George E. Cartwright, Medical Corps, Army of the United States, and Maxwell M. Wintrobe, M.D., Salt Lake City, Utah ---------- 886

Absorption and Excretion of Sulfathiadiazole and Sulfamethylthiadiazole After Oral Administration in Human Beings. Manson Meads, M.D., and Maxwell Finland, M.D., Boston, Mass. ---------- 900

The Quantitative Determination of the Thymol Turbidity Reaction of Serum. Allyn B. Ley, M.D., Jessica H. Lewis, M.D., and Charles S. Davidson, M.D., C.M., Boston, Mass. ---------- 910

(Contents continued on inside cover)

PUBLISHED BY THE C. V. MOSBY COMPANY, 3207 WASHINGTON BLVD., ST. LOUIS 3

Copyright 1946 by The C. V. Mosby Company

Laboratory Methods

Semiquantitative Estimation of Bilirubin in the Urine by Means of the Barium-Strip Modification of Harrison's Test. C. J. Watson and Violet Hawkinson, Minneapolis, Minn. ---- 914

Applicability of the Biuret Reaction to the Determination of Serum Albumin by Methanol Precipitation. Halvor N. Christensen, Ph.D., Cooperstown, N. Y. -- 916

Oxygen Consumption and Drug Action: A Method for Measurement of the Respiration of Aquatic Animals. W. A. Robbie, Ph.D., and P. J. Leinfelder, M.D., Iowa City, Iowa ---- 918

The Methylene Blue Test for Bilrubinuria: Clinical and Spectrophotometric Observations. Gordon D. Stokes, M.D., Earl E. Gambill, M.D., and Arnold E. Osterberg, Ph.D., Rochester, Minn. ---- 924

A Simple Method of Determining the Specific Gravity of Small Samples of Urine. Lee Monroe, M.D., and James Hopper, Jr., M.D., San Francisco, Calif. ---- 934

Book Reviews

Book Reviews ---- 936

Editor

CARL V. MOORE, M.D.
Washington University School of Medicine
600 South Kingshighway
St. Louis 10, Mo.

Editorial Board

CLIFFORD J. BARBORKA, M.D.
Northwestern University, Chicago

PAUL R. CANNON, M.D.
University of Chicago, Chicago

RUSSELL L. HADEN, M.D.
Cleveland Clinic, Cleveland

GEORGE HERRMANN, M.D.
University of Texas, Galveston

JOHN A. KOLMER, M.D.
Temple University, Philadelphia

W. C. MacCARTY, M.D.
Mayo Clinic, Rochester, Minn.

T. B. MAGATH, M.D.
Mayo Clinic, Rochester, Minn.

EDWARD MUNTWYLER, Ph.D.
Long Island College of Medicine, Brooklyn

VICTOR C. MYERS, Ph.D.
Western Reserve University, Cleveland

M. H. SOULE, Sc.D.
University of Michigan, Ann Arbor

FREDERICK J. STARE, M.D.
Harvard University, Boston

ARNOLD D. WELCH, Ph.D., M.D.
Western Reserve University, Cleveland

Vol. 31, No. 8, August, 1946. The Journal of Laboratory and Clinical Medicine is published monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis (3), Mo. Subscription Price: United States, its Possessions, Pan-American Countries, $8.50 per year; Canada, $9.50; Foreign, $9.50. Entered as Second-Class Matter October 8, 1915, at the Post Office at St. Louis, Missouri, under Act of March 3, 1879. Printed in the U. S. A.

THE ELIMINATION OF HUMAN REACTION-PRODUCING MATERIAL FROM HORSE-DERIVED ANTISERUMS: AN ELECTRICAL METHOD

EUGENE CARDONE
NEW YORK, N. Y.

INTRODUCTION

IMMUNE serum therapy in man has been associated with certain serum reactions[1, 2] that have done much to discourage its use in the patient.

The causes for these reactions have not been completely eliminated from sera by purification and concentration. These efforts have ranged from heating to complex chemical protein fractionations.[3, 4] The aim, in general, has been to eliminate all the immunologically inert protein from sera in the hope that the reaction-producing substances would go with them. The remaining antibody proteins are concentrated to increase the potency of the antibody per unit of volume. A smaller dose is then possible.

More recently the possibility that bacterial contaminants may produce pyrogen for man[5, 6] has spurred the preparation of immune sera under sterile conditions.

In our laboratory we have purified antibacterial sera by eliminating an acid fraction of protein. The antibody-bearing globulin has been collected for concentration by water precipitation.[7] This method has been improved upon from time to time,[8, 9] but the products so obtained still cause untoward reactions upon intravenous injection in man.

The electrical method to be described herein was used chiefly to reprocess pneumococcic antiserum preparations that had been shown to contain immediate reaction-producing material by clinical test. They had been purified and concentrated with the water precipitation method.[7] After the electrical reprocess they were again tested clinically to determine to what extent the reaction-producing material had been eliminated.

APPARATUS

The apparatus (Fig. 1) consists of six electrodes, each made up of a zinc jacket filled with tap water and with a platinum wire disposed in its center. The latter is insulated from the zinc jacket by a rubber plug at the top and a rubber washer at the bottom.

Cellulose casings,* with a diameter and length suitable to the size of the apparatus, are cut and one end is wet in water. While wet, this end is constricted manually by folding and is sealed by wrapping one or two rubber bands tightly around it.

From the William Hallock Park Laboratory, Bureau of Laboratories, Department of Health, New York City.
Received for publication, Sept. 24, 1945.
*Bought from the Visking Cellulose Casing Corp., Chicago, Ill.

For best results the casings must be large enough to contain one-seventh the volume of diluted serum to be processed. When they have been filled with this volume of tap water, the zinc jacket electrodes are immersed in each one. The semipermeable membranes are supported in place by round metal frames to which they are secured. These are fastened by means of screws to a glass top which fits snugly over the vat containing the serum. The zinc jacket electrodes are also supported by the glass top through holes cut in it, and each is suspended in the center of the cup formed by the cellulose casing membrane. These metal electrodes are immersed in the water contained in the membranes to at least two-thirds of their length. Two electrically driven stirrers inserted through holes in the glass top complete the apparatus.

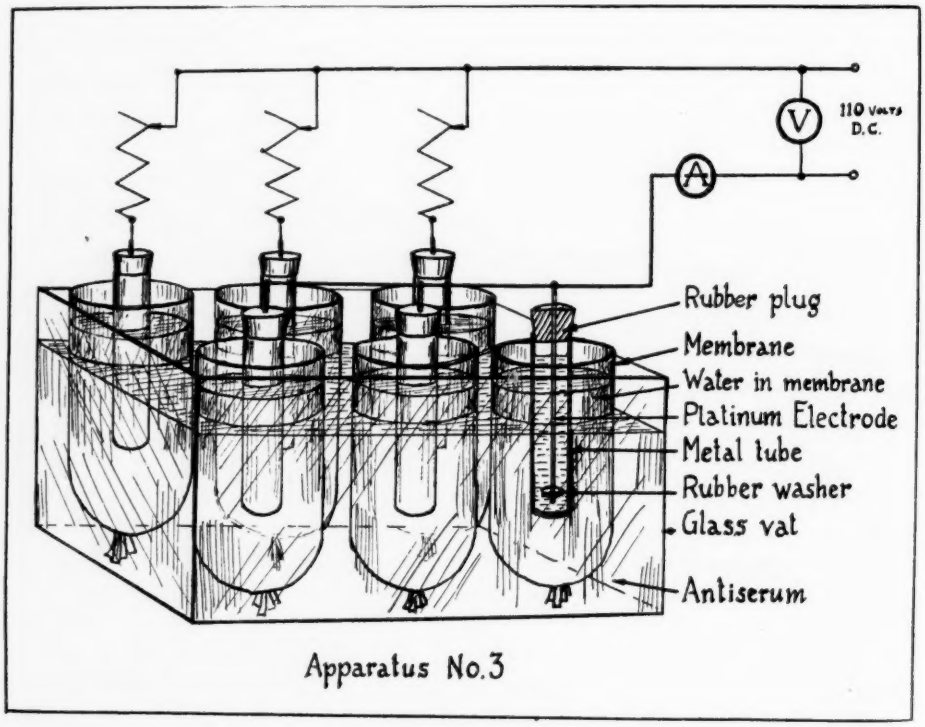

Fig. 1.

PROCEDURE

The serum* to be processed is adjusted to pH 5.1 with normal acetic acid. It is diluted in the proportion of one volume to three and one-half volumes of distilled water freed of carbon dioxide gas by boiling. Solid sodium chloride is added to give a final concentration of one-half of one per cent. The material is poured into the vat of the apparatus. A 110-volt direct current is turned on and by means of series resistances is adjusted to 60 ma. with an electromotive force of approximately 90 volts.

*Our concentrated antibacterial sera contain about 13 mg. N/Gm. of serum.

The pH value is determined at intervals. When it reaches pH 7.2 the electrical process is stopped, and the serum is removed from the vat. At this point all the globulin proteins are precipitated. The pH is adjusted to 5.1 with normal acetic acid; the serum is placed in cellophane casings 6 inches in diameter and 24 inches long (1½ liters per casing) and dialyzed for six days in a running water bath at 15° C. with moderate agitation. At the end of six days the reaction of the dialysate is between pH 5.7 and 6.3. The reaction is brought to pH 5.1 with normal acetic acid. Saturated ammonium sulfate solution is added slowly and with constant stirring to 30 per cent saturation. The pH is adjusted to 5.9 with normal sodium hydroxide, and the serum is left at room temperature overnight. The clear supernatant fluid is siphoned off, and the precipitate is centrifuged. The supernatant fluid is added to the material siphoned off, and the precipitate is discarded. Saturated ammonium sulfate solution is again added to 50 per cent saturation. After one hour at room temperature the solution is filtered through hardened paper. The filtrate is discarded, and the precipitate is thoroughly dried by pressing between pads of absorbent paper. The precipitate is then dialyzed until free of sulfate ion. If the nitrogen content of the dialysate is higher than desired, it is diluted to a suitable value with distilled water. Solid sodium chloride to a concentration of 1 per cent and suitable preservatives are added. The pH is adjusted to 7.4 with normal sodium hydroxide, and the solution is left in the cold overnight.

The treated serum is clarified by centrifugation. It is then filtered through a Berkefeld stone to sterilize it, and its potency is determined.

CLINICAL RESULTS

During the development of the chemical procedure following the electrical process, it became evident that when the sera were not dialyzed or were dialyzed insufficiently after the electrical preparation they did not lose reaction material at all or they lost it in proportion to the degree of completion of dialysis. These data are presented in Table I.

Type 1, Preparation 107, was clinically free from reactions before and after reconcentration. However, it had been found to contain toxic material for the rabbit which was lost after the electrical preparation and six and a half days of dialysis. Type 8, Preparation 23, had been deprived of the important last day of dialysis.* Upon clinical trial this preparation was still found to contain considerable reactive material.

The remaining preparations recorded in Table I had been dialyzed for four days or less following the electrical treatment. They show little or no improvement in the nature of the reactions caused in man or in the rabbit.

That dialysis of sera without electrical preparation does not eliminate reaction-producing material from them was proved in our laboratory over a period of many years. Serum lots, prepared regularly,[7, 9] that had been shown to contain reactive material by clinical trial were reprocessed by different chemical procedures in an effort to develop one that would eliminate this material.

*On this day more reactive material is eliminated than on earlier days, because it is not hampered by other impurities with faster rates of dialysis.

TABLE I. REACTION-PRODUCING MATERIAL, MADE DIALYZABLE BY ELECTRICAL PREPARATION, NOT ELIMINATED COMPLETELY BY LESS THAN SIX DAYS OF DIALYSIS

RECONCENTRATED PNEUMOCOCCIC ANTISERUM		POTENCY/C.C. (MOUSE UNITS) ANTISERUM		NITROGEN VALUES (MG. N/GM. OF SERUM) ANTISERUM		RABBIT TOXICITY TEST[11, 12] (° F.) ANTISERUM		RESULTS OF CLINICAL TRIAL*		DAYS OF DIALYSIS AFTER ELECTRICAL PREPARATION
TYPE	PREPARATION NO.	BEFORE RECONCENTRATION	AFTER RECONCENTRATION	BEFORE RECONCENTRATION	AFTER RECONCENTRATION	TEMPERATURE RISE BEFORE RECONCENTRATION	TEMPERATURE RISE AFTER RECONCENTRATION	ANTISERUM BEFORE RECONCENTRATION	ANTISERUM AFTER RECONCENTRATION	
1	107	5058	4012	14.53	13.53	1.9	0.3	Acceptable	Acceptable	6.5
8	23	3000	3000	13.7	13.4	Not done	1.3	17 treated % No reactions 35 Dyspnea 53 Chill 6 Urticaria 6 Nausea and vomiting 12 Blood pressure drop 6	7 treated % No reactions 53 Dyspnea 14 Chills 0 Urticaria 14 Vomiting 14 Blood pressure drop 0	5.0
18	17	3700	3493	10.9	9.05	1.5	1.4	Reactions	Not done; toxic for rabbits	4.0
18	19	6212	9312	13.0	11.7	2.4	2.5	Reactions	Not done; toxic for rabbits	3.5
5	34	3756	2390	14.55	9.95	2.3	1.9	Reactions	Not done; toxic for rabbits	3.0

8	39	2400	3100	12.2	15.15	2.1	0.9	Not done; toxic for rabbits	Gave chill in one case treated	2.5
8	40	1544	3078	11.3	12.9	2.4	1.9	Reactions	Not done; toxic for rabbits	2.5
8	44	2307	1983	13.2	11.35	2.3	1.2	Reactions	Not done; toxic for rabbits	2.5
5	23	2817	2970	12.7	13.85	1.9	2.5	12 treated %	3 treated %	1.5
								No reactions 33	No reactions 33	
								Dyspnea 33	Dyspnea 0	
								Chills 17	Chills 33	
								Urticaria 33	Urticaria 0	
								Nausea 17	Temperature rise 33	
								Headache 8	Vomiting 33	
									Pain in chest 33	
1	79	3000	3000	11.75	14.05	2.3	1.6	Preparations pooled for this common original had caused shock, chills, dyspnea, and urticaria	2 treated %	0
1	80		6000		13.35		1.8		No reactions 0	
									Dyspnea 50	
									Chills 100	
									Urticaria 100	
									3 treated %	2
									No reactions 33	
									Dyspnea 0	
									Chills 33	
									Urticaria 66	

*Most of these preparations had been tested clinically before the rabbit test had been adopted as the basis for determining whether or not a serum was to be tested clinically.

TABLE II. REACTION-PRODUCING MATERIAL, MADE DIALYZABLE BY ELECTRICAL PREPARATION, ELIMINATED BY SIX DAYS OF DIALYSIS

PNEUMOCOCCIC ANTISERUM		POTENCY/C.C. (MOUSE UNITS)		NITROGEN VALUES (MG. N/GM. OF SERUM)		RABBIT TOXICITY TESTS[11, 12]* (° F.)		RESULTS OF CLINICAL TRIAL	
		ANTISERUM		ANTISERUM		ANTISERUM		ANTISERUM	
TYPE	PREPARATION NO.	BEFORE RECONCENTRATION	AFTER RECONCENTRATION	BEFORE RECONCENTRATION	AFTER RECONCENTRATION	TEMPERATURE RISE BEFORE RECONCENTRATION	TEMPERATURE RISE AFTER RECONCENTRATION	BEFORE RECONCENTRATION	AFTER RECONCENTRATION
2	83	4387	6450	14.35	10.9	1.8	0.2	All had caused immediate serum reactions	Acceptable†
1	88	5050	5600	12.85	10.7	0.8	0.1		Acceptable
5	31	5040	5850	12.75	15.45	2.0	1.0		Acceptable
4	38	4775	3750	12.85	10.8	1.7	0.2		Acceptable
5	35	2953	3680	13.3	13.1	2.2	1.0		Acceptable
8	45	2990	2490	12.85	12.85	2.1	0.3		Acceptable
7	34	1200	2167	11.4	15.25	2.0	0.8		No clinical test report available
4	36	1750	2100	12.75	10.25	2.7	0.5		
2	92	1680	1430	14.65	12.0	1.7	0.7		
1	98	2612	2087	12.6	13.7	1.1	0.5		
8	43	3423	3553	13.5	12.9	3.0	0.4		
18	18	8800	6280	13.2	11.75	1.5	0.5		

*An antiserum that causes an average temperature rise higher than 1° F. in three rabbits[11, 12] may cause reactions if injected intravenously in man. When the rabbit temperature rise is 1° F. or less the antiserum is considered unlikely to cause reactions in man.

†An "acceptable" serum caused either no reactions upon intravenous injection in man or the reactions were of such a mild nature that the serum was considered satisfactory for distribution.

TABLE III. ELECTRICAL METHOD COMPARED TO THREE OTHER METHODS IN ELIMINATION OF IMMEDIATE REACTION-PRODUCING MATERIAL FROM A COMMON ORIGINAL ANTISERUM

ANTISERUM		METHOD OF PREPARATION	RESULTS OF CLINICAL TRIAL	RABBIT TOXICITY TEST[11, 12] (° F.)
TYPE	PREPARATION NO.			
Common original, 5	22	Prepared with regular method[7, 9]; this serum had been dialyzed for 6 days	4 treated 3, chills 1, chilly sensation 2, temperature rise 1, vomiting	2.0
5	32	Used ⅓ of common original; serum heated to 56° C. for ½ hour and adsorbed with kaolin according to technique of Goodner and associates[11]; globulin, precipitated by 50% saturation with $(NH_4)_2SO_4$, dialyzed for 6 days before use	Not tested clinically because too low in potency and because the proteins had become irreversibly insoluble, probably due to heating	0.7
5	33	Used ⅓ of common original; fraction of protein precipitated up to 30% saturation with $(NH_4)_2SO_4$ discarded; fraction precipitated up to 50% $(NH_4)_2SO_4$ saturation dialyzed for 6 days before use	Not tested clinically because toxic for rabbits	1.3
5	31	Used ⅓ of common original; Prepared by electrical method with 6 days of dialysis	3 treated 3, no reactions	1.0

After each effort the serum was dialyzed for six days. Upon clinical retest these sera still caused immediate serum reactions in man.

The serum preparations recorded in Table II were made from regularly prepared lots[7, 9] that had been shown to contain reactive material for man upon clinical trial. Furthermore, all but one gave more than the acceptable temperature rise in the rabbit test for pyrogen. Each was processed electrically and then dialyzed for six days. Upon test it was found that all had been cleared of significant pyrogenic material for the rabbit, and those preparations that it was possible to test clinically had lost the power to cause immediate serum reactions in man.

In Table III is presented the comparison in power to eliminate human and rabbit reaction material from pneumonia horse sera by the electrical procedure and six days of dialysis and three other methods of preparation also followed by six days of dialysis. The common original used for these preparations was an antiserum which had been prepared with the water precipitation method[7, 9] and which had caused immediate serum reactions in man when tested clinically. The electrical method eliminated the reactive material more effectively.

RESULTS OF THIS METHOD WITH OTHER BIOLOGICALS

Other biologic products have been reprocessed with this method to improve them.[10] In Table IV are shown the results of its adaptation to the reconcentration of antimeningococcus sera prepared by the water precipitation method.[7, 9] The potency per unit of protein nitrogen was increased, allowing smaller injections and thus rendering the preparations more suitable for human use.

In other experiments antiscarlatinal globulin reprocessed by this method was tested by Dr. Samuel Karelitz in patients at the Willard Parker Hospital. It was found that the incidence of delayed serum sickness in the sixty-four patients treated with this material was about 38 per cent. More significant, however, was the unusually mild nature of these reactions as compared with those caused by regular material.*

TABLE IV. MENINGOCOCCUS ANTISERUM MADE MORE POTENT PER UNIT OF PROTEIN NITROGEN BY ELECTRICAL PROCESS

REGULARLY PREPARED[7, 9] PREPARATION NO.	ELECTRICAL RECONCENTRATION PREPARATION NO.	AGGLUTINATION TEST* TYPE AND ANTIGEN NO. USED				OTHER TESTS*			MG. N/GM. OF SERUM
		I	I	III	II	AGAR PLATE PRECIPITATION	S-I PRECIPITATION	MOUSE PROTECTIVE RATIO	
		331	1027	302	36				
77		2.0	2.0	2.0	2.0	++++	1-512	336%	11.7
	77S	2.0	2.0	2.5	16.0	++++++	1-2048	653%	11.65
79		1.5	1.5	2.0	8.0	+++	1-225	225%	9.75
	79S	2.0	1.0	0.6	8.0	+++	1-512	157%	6.55
72		1.2	2.0	2.0	16.0	++++	1-1024	184%	11.3
	72S	1.2	2.0	2.0	16.0	++++	1-1024	186%	6.8
N. I. H. Control		1.0	1.0	1.0	1.0	+++	1-512	100%	–

*The potencies were determined by Mrs. C. R. Falk of the New York City Department of Health.

*Personal correspondence from Dr. Samuel Karelitz.

DISCUSSION

Whether the reaction-producing material is adsorbed on the surface or whether it is a more integral part of the large molecular aggregate which is the antibody entity, it is freed by the electrical process. Whether it is of organic or of inorganic origin, it is in a form, or it is changed to a form, that is eliminated or inactivated by simple dialysis.

SUMMARY

An electrical process is described which, when followed by six days of dialysis, has eliminated from purified and concentrated horse-derived antisera substances which had caused immediate serum reactions when injected intravenously in man.

In most lots tested it was possible to remove reaction-producing material by this method without lowering the antibody potency per unit of protein nitrogen. Indeed, in the majority of lots the potency was increased.

The author is indebted to Dr. Wheelan D. Sutliff and Miss Annabel W. Walter of the Pneumonia Control Division, for their valuable cooperation in carrying out potency determinations and rabbit tests and in supplying results of clinical trial as indications of the presence or absence of reaction-producing material.

REFERENCES

1. Rutstein, D. D., Reed, E. A., Langmuir, A. D., and Rogers, E. S.: Immediate Serum Reactions in Man; Classification and Analysis of Reactions to Intravenous Administration of Antipneumococcus Horse Serum in Cases of Pneumonia, Arch. Int. Med. **68**: 25, 1941.
2. Bullowa, J. G. M.: The Management of the Pneumonias, New York, 1937, Oxford University Press, p. 317.
3. Felton, L. D., and Kauffmann, G.: The Pyrogenic Reaction to Concentrated Antipneumococcus Serums, With Respect to Certain Serum Constituents, J. Infect. Dis. **49**: 337, 1931.
4. Sabin, A. B., and Wallace, G. B.: On the Nature of Chill-Producing Principle in Antipneumococcus Serum, J. Exper. Med. **53**: 339, 1931.
5. Co Tui, and Schrift, M. H.: Production of Pyrogen in Sera by Bacteria, Proc. Soc. Exper. Biol. & Med. **42**: 549, 1939.
6. Robinson, E. S., Flusser, B. A.: Studies on Pyrogens; Isolation of Pyrogens From Various Microorganisms, J. Biol. Chem. **153**: 529, 1944.
7. Klein, A. J., Greenwald, C. K., and Falk, K. G.: The Refining of Antiserums, J. Lab. & Clin. Med. **19**: 1324, 1934.
8. Cardone, E., and Mazzarella, R.: The Adsorption of Phenol by Protein in Preparation of Certain Biologic Products, J. Lab. & Clin. Med. **24**: 137, 1938.
9. Cardone, E., and Falk, K. G.: The Refining of Antisera, J. Lab. & Clin. Med. **24**: 417, 1939.
10. Cardone, E.: An Electrical Procedure for the Repurification-Concentration of Antitoxin, J. Lab. & Clin. Med. **28**: 1126, 1943.
11. Goodner, K., Horsfall, F. L., Jr., and Dubos, R. J.: Type-Specific Antipneumococcic Rabbit Serum for Therapeutic Purposes; Production, Processing, and Standardization, J. Immunol. **33**: 279, 1937.
12. Beattie, M. W., Berlow, A., and Leshne, F. L.: Rabbit Test for Thermal Reactions Caused by Concentrated Horse Serum, Proc. Soc. Exper. Biol. & Med. **46**: 453, 1941.

A STUDY OF ABNORMAL T WAVES IN PATIENTS PRESENTING NO EVIDENCE OF ORGANIC HEART DISEASE

ISIDORE STEIN, M.D.
BROOKLYN, N. Y.

EVER since Einthoven introduced the electrocardiogram, the interpretation and significance of the T wave has been a matter of much discussion. We still do not know the exact part the T wave plays in the electrodynamics of the cardiac cycle. The prevalent opinion is that the T wave represents the recession of the electrical impulse in the ventricles. According to Mines, as quoted by Wilson and Finch,[1] the form of the T wave depends on the order in which various regions of the ventricular muscle complete their electric activity. If the apex of the left ventricle returns to the resting state in advance of the base of the right ventricle, T is positive; if the reverse occurs, the T wave is negative.

The normal T wave is too well known to bear description at this time. Low voltage, biphasic, or inverted T waves in the first, second, or precordial leads are considered to be abnormal. In children or in pregnant women these aberrations in the T wave in the fourth lead are normal. The present paper does not include patients of this type.

The present study was made among a group of soldiers at a large Army hospital. Most of the subjects were from 20 to 40 years of age; some were older, and a few young women were included. The patients varied in body build in all extremes. Some presented numerous complaints suggestive of the so-called functional cardiac disorders; others appeared for examination only as a routine procedure. They presented two factors in common: (1) abnormality of the T waves in the electrocardiogram (there were no associated R-T or S-T segment changes); (2) none presented any other evidence of organic heart disease, as could be discovered by complete physical examination in which auscultation of the heart was performed in the upright, recumbent, and left lateral positions prior to and following exercise (hopping on each leg fifty times). Roentgen and fluoroscopic heart studies and sedimentation rate determinations were within the normal range except for the sedimentation rate in Patient 25 (Table I) whose case history will be taken up in greater detail later.

The electrocardiograms were obtained in the recumbent position and were taken at frequent intervals both before and after exercise (Figs. 1 to 5).

From November, 1943, to November, 1945, 5,520 electrocardiograms were taken at this hospital on 4,810 patients. Of these, fifty-one, or 1.0 per cent, showed significant T-wave changes, twenty-three predominantly in the chest lead (CR_4) (Table II), and twenty-eight showed the deviations in the limb leads (Table I) in individuals without any other evidence of heart disease.

Received for publication, March 12, 1946.

TABLE I. LIMB LEADS

PATIENT	AGE	SEX	BODY BUILD	DIAGNOSIS	ABNORMAL T WAVES	EFFECT OF EXERCISE
1	22	M		Psychoneurosis	II, III, low voltage	Normal
2	26	M		Psychoneurosis	II, low voltage	Normal
3	40	M	Medium	Psychoneurosis	II, low voltage	Normal
4	24	M	Medium	Psychoneurosis	II, isoelectric	Normal
5	26	M	Slender	Psychoneurosis (?)	I, low voltage	Normal
6	25	M	Medim	Psychoneurosis	I, II, low voltage	Normal
7	25	M		Transient hypertension	I, II, III, low voltage	Normal
8	30	M	Medium	No disease	II, inverted	Low voltage
9	29	M	Medium	Arthritis of feet (?)	II, inverted	Low voltage
10	35	M		Psychoneuosis	II, isoelectric	Normal
11	19	M	Medium	Psychoneurosis	II, inverted	Normal
12	29	M		Psychoneurosis; nontoxic adenoma of thyroid	II, isoelectric	Normal
13	36	M	Husky	Postinfluenzal asthenia (?)	II, III, inverted; II, low voltage (two days later)	None
14	32	M		Psychoneurosis	II, III, inverted	None
15	20	M	Medium	No disease	II, III, inverted	None
16	23	M	Slender	Psychoneurosis	II, III, inverted	Normal
17	23	M	Medium	No disease	II, III, inverted (normal six days later)	II, III, normal; P-R, .28 second (see Fig. 8)
18	24	M	Husky	Psychoneurosis	II, inverted (normal three weeks later)	Normal
19	47	M	Husky	Transient intestinal obstuction	II, biphasic; III, inverted	Normal
20	27	M	Medium	Fracture (leg)	II, III, inverted	None
21	22	M	Medium	Rheumatoid arthritis (?)	II, isoelectric; III, inverted (normal three days later)	Normal
22	29	M	Medium	Psychoneurosis	II, low voltage (normal two days later)	Normal
23	37	M	Medium	Psychoneurosis	I, II, biphasic	Normal
24	26	M	Medium	Psychoneurosis (?)	II, inverted	Normal
25	29	M	Husky	Syphilis; psychoneurosis	I, transient inversion; II, III, inverted (see Fig. 4)	None
26	30	M	Medium	Psychoneurosis	II, III, inverted	None
27	23	M	Medium	Rheumatoid arthritis (?)	I, isoelectric; II, low voltage; I, low voltage; II, normal ten days later	Not performed
28	33	M	Husky	Psychoneurosis	II, isoelectric	Normal

TABLE II. LEAD IV (CR_4)

PATIENT	AGE	SEX	BODY BUILD	DIAGNOSIS	T WAVES	EFFECT OF EXERCISE
1	29	M	Medium	Psychoneurosis	IV, inverted	IV, upright
2	19	M		Arthritis (not rheumatic fever); psychoneurosis	IV, isoelectric	IV, upright
3	20	M	Slender	Psychoneurosis	IV, inverted	IV, upright
4	37	M		Psychoneurosis	IV, biphasic	IV, upright
5	32	F	Medium	Psychoneurosis	IV, inverted	IV, upright
6	30	M	Husky	Psychoneurosis	IV, isoelectric	IV, upright
7	26	F	Slender	Psychoneurosis	IV, inverted	IV, upright
8	35	M	Medium	Psychoneurosis	IV, biphasic	IV, upright
9	22	M	Slender	Unknown	IV, inverted	No change
10	35	M		Psychoneurosis	IV, inverted	IV, upright
11	23	M	Medium	Psychoneurosis	IV, biphasic	IV, upright
12	27	M		Schizophrenia	IV, inverted	IV, upright
13	39	M	Medium	Tracheitis	IV, inverted	IV, upright
14	41	M	Medium	Peptic ulcer	IV, inverted	IV, upright
15	31	M	Medium	Bronchitis	IV, inverted	IV, upright
16	33	M	Medium	Meningitis	IV, low voltage	IV, voltage higher
17	42	M		Herniorrhaphy	IV, biphasic	IV, upright
18	48	M	Husky	Nasal polyps and sinusitis	IV, biphasic	IV, upright
19	32	F	Medium	Extrasystoles	IV, isoelectric	IV, upright
20	36	F	Medium	No disease	IV, inverted	IV, upright
21	21	M		No disease	IV, inverted	IV, upright
22	20	M	Medium	No disease	IV, inverted	IV, upright
23	49	M	Slender	No disease	IV, biphasic	IV, upright

ANALYSIS OF RESULTS

Age and Sex.—The age of our patients varied between 19 and 47, the average being 29.9 years. All but four of the group were men.

Body Constitution.—A record of the body constitution was not made in all instances. In forty patients where a notation was made, eight were of the stocky broad-chested type, six belonged to the tall slender group, while the largest majority (twenty-six) were of medium build.

Diagnosis.—The greatest number of our patients was in the psychoneurotic group. Twenty-nine, or 57 per cent, were included under this heading (one individual suffered with schizophrenia). In addition, one patient had psychoneurosis with a nontoxic adenoma of the thyroid gland; another had psychoneurosis and adequately treated latent asymptomatic syphilis with no physical signs pointing to this condition. Three patients had arthritis (not rheumatic fever); one had a postinfluenzal asthenia; another had a transient intestinal obstruction (the cardiac study was made during his convalescence); and one patient had a fracture of his leg. There were, in addition, single cases of peptic ulcer, bronchitis, tracheitis, convalescent meningitis, hernia, extrasystoles, and nasal polyps. Seven patients failed to show evidence of any disease whatever.

Leads Involved.—In the patients showing the abnormalities of the T waves in the limb leads, the involvement in the majority (twelve) was in the sec-

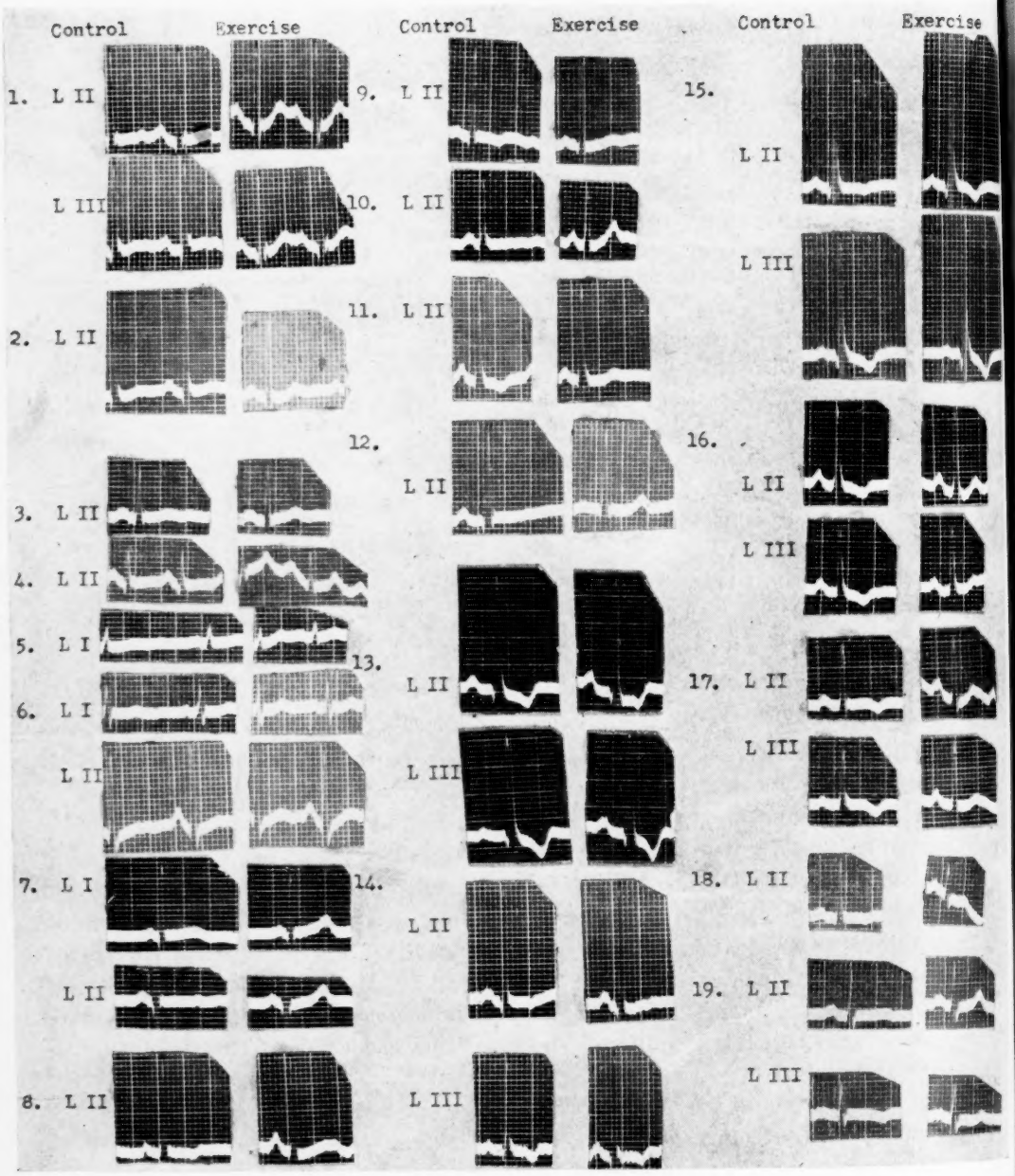

Fig. 1.—Limb leads.

ond lead. In eleven, Leads II and III showed the T-wave changes, three occurred in Leads I and II, one in Lead I alone, and one individual had abnormal T waves in the three limb leads.

There were twenty-three patients whose T_4 was abnormal. Of this group four also showed similar deviations in the T wave in the first lead. In one it occurred in combination with abnormal T_2 and T_3. In one patient the T waves in all four leads were involved.

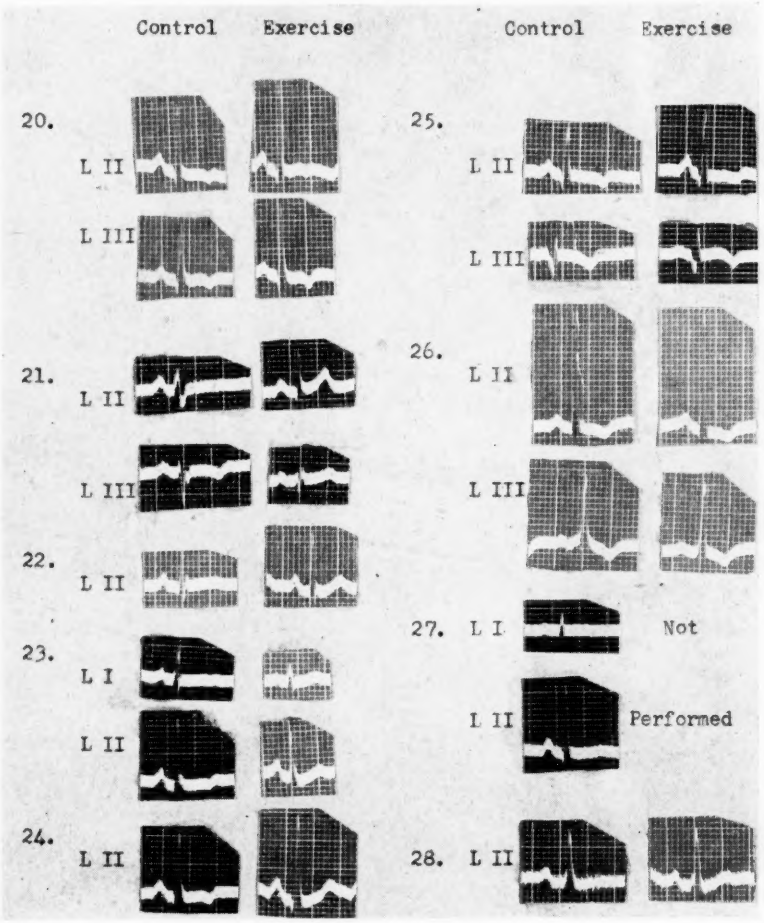

Fig. 2.—Limb leads.

Response to Exercise.—All but one of the patients were subjected to a standard amount of exercise (hopping on each leg fifty times) immediately following which an electrocardiogram was taken, again in the recumbent position. In those with the limb lead changes twenty-one revealed a reversal of the T wave to the upright position (Figs. 1 and 2). Six of the patients showed no change following this procedure.

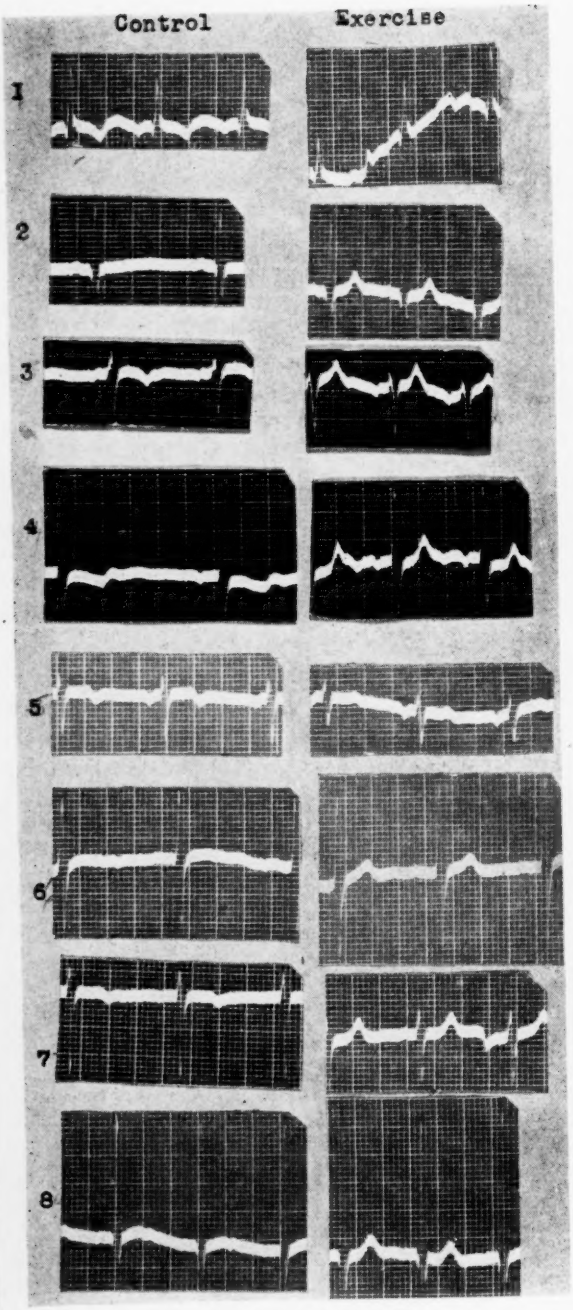

Fig. 3.—Lead IV (CR_4).

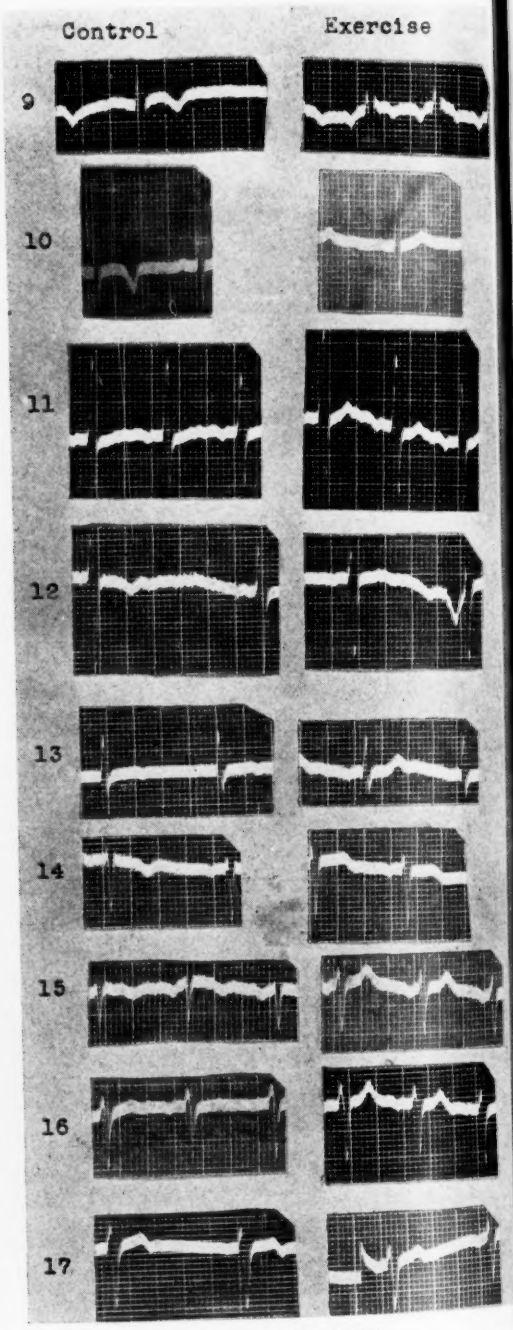

Fig. 4.—Lead IV (CR_4).

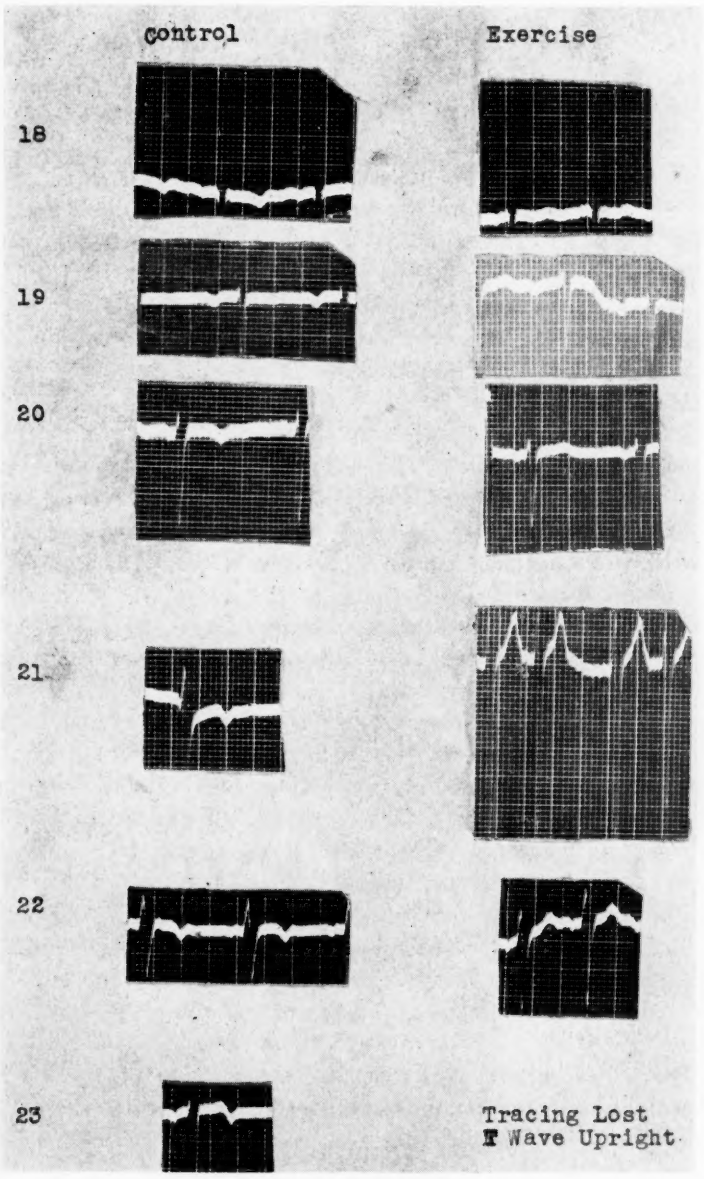

Fig. 5.—Lead IV (CR_4).

In those with the T_4 deviations the effects were even more striking; twenty-two of the twenty-three revealed a return to a normal T wave (Figs 3 to 5). In the entire group of fifty patients forty-three, or 86 per cent, showed a reversal of an abnormal to a normal T wave following physical exercise.

Heart Rates.—The ventricular rates in the limb lead group ranged between 50 and 138 per minute. Sixteen patients were below 90 per minute while twelve showed higher figures.

Among the T_4 group the largest number (six) had a ventricular rate between 70 and 79, four between 80 and 89, and four between 90 and 99. Seven patients had a rate between 50 and 69. Only six were above 90, and of these only two exceeded a rate of 100 beats per minute.

In summary, thirty-three of our patients, or 65 per cent of the group, had a ventricular rate under 90 per minute. This would tend to eliminate tachycardia as a factor in the production of the T-wave changes.

DISCUSSION

Current criteria for the normal electrocardiogram were published by Lewis and Gilder,[2] in 1912, and were based on a study of fifty-three young men. This field was left unexplored for many years, and no extensive investigation of the normal was undertaken until 1926 when Ferguson and O'Connell[3] reported a series of electrocardiograms in 1,812 midshipmen at Annapolis without any evidence of heart disease (with three exceptions). There were eight instances where T_2 was inverted, diphasic, or isoelectric. Some of these were normal when a second tracing was taken. The mobilization of our Armed Forces during the war period gave the medical profession the golden opportunity of examining millions of young men, among which there were many thousands upon whom routine electrocardiographic studies were made. In this manner it became evident that bizarre cardiographic patterns usually indicative of grave myocardial disease were too frequently observed in young individuals devoid of any organic stigmas.

Hall, Steward, and Manning[4] studied a group of 2,000 "normal" aviators and found nine with electrocardiograms of an abnormal pattern. Included among these were negative T_1 or T_2 or both and bundle branch block. Graybiel and associates[5] reviewed the findings in 1,000 young, healthy aviators and discovered two with inverted T waves in the second lead but none in the first or fourth leads. T_2 in one was biphasic.

Our results in fifty-one patients constitute an addition to the list of electrocardiographic abnormalities in "normal" young people (twenty-eight involving limb leads, twenty-three with chest lead changes).

It is evident therefore that abnormalities of the T wave in "normal" individuals occur with fair incidence. Not so clear, however, is the mode of production of this aberration in the electrocardiogram and the significance, if any, to be attached to this phenomenon. It may be argued that the unusual T wave is the first indication of heart disease in these people, and disabling

signs or symptoms are sure to appear in the future. This opinion cannot be refuted in toto until these patients have been followed over a number of years; however, such a grave prognosis seems very unlikely.

Yet, if we are to base these changes on functional grounds, what are the factors involved? It is well known that the height of T waves is lowered or that the T waves can become inverted when the subject's position is changed from the recumbent to the sitting or standing attitude.[6-8] Our patients were all recumbent when their tracings were taken. The location of the diaphragm is presumed to be a factor involved in these aberrations. High diaphragms can be expected in the sthenic type of patient and a lower position in those of the slender habitus. In our series this would not be applicable, as we encountered all types of body build, the largest group (twenty-six) being made up of those of medium habitus.

Imbalance of the autonomic nervous system has been suggested as an element in the production of unusual T waves. Wendkos[9] studied a group of soldiers and concluded that the T-wave changes could be attributed to predominance of either vagal or sympathetic tone. Graybiel and White[10] found inverted T waves in Leads II in seven instances of neurocirculatory asthenia.

By far the largest number of our series (60 per cent) fall in the "nervous" group who demonstrated unequivocal signs of derangement of the autonomic nervous system. To attribute the faulty T waves in these individuals to sympathetic or parasympathetic imbalance seems rational. The attempt to affect this system artificially through the medium of standardized exercise brought a restitution of the normal T waves in 86 per cent of our patients. This finding lends support to the suggestion that we were dealing basically with a functional situation.

We do not feel that emotional factors at the time the electrocardiographic examination was undertaken played any part in our findings. No outward apprehension was noted during this period and, as has been noted, tachycardia was not a dominant feature in our patients. Thirty-three of our group had ventricular rates of 90 per minute or less while eighteen were above that figure. A "fear reaction"[11] was not observed at any time and played no part in the picture presented here.

When one considers that seven of our patients showed no evidence of any disease process whatever and that thirteen others were suffering from ailments (nonrheumatic arthritis, transient intestinal obstruction, a fracture) unrelated to the heart, it is conceivable that we are merely dealing with a physiologic variant and that our criteria of the "normal" must undergo reconsideration. It is plausible that the wide range of the normal electrocardiogram has not been adequately explored. This would seem reasonable when we remember that the standards originally established by Lewis and Gilder[2] were based on the study of only fifty-three individuals. This statement is supported by the fact that in six of our patients (Patients 13, 17, 18, 21, 22, 27, Table I) tracings taken anywhere from two hours to ten days later showed a spontaneous reversal of the T wave to normal (Fig. 6). Similar findings were recorded

by Ferguson and O'Connell.[3] Dupuy[12] advances such a thought in a presentation of five cases with T-wave abnormalities in other leads than in the third.

The value of exercise or sympathetic stimulation in the differential diagnosis of organic or functional heart disease remains a matter for further study. Without a control group of patients known to have organic heart disease, no conclusions are warranted.

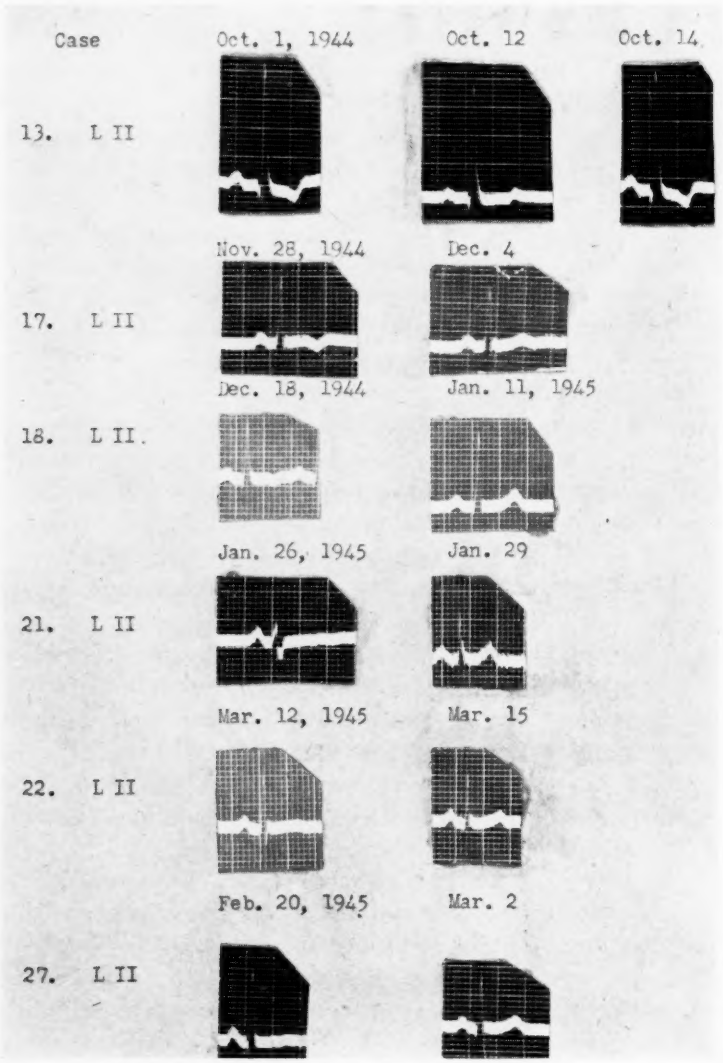

Fig. 6.—Spontaneous reversals of T waves.

The case history of one patient (Patient 25, Table I) is described in more detail because of several unusual features. This man was 29 years of age and entered the hospital because of frequent bouts of sharp pains in his chest for the previous two years. These pains were noted while at complete rest and

at other times during exercise; they lasted for about twenty minutes and were occasionally accompanied by "hard breathing." In the patient's words, he was unable "to get in enough air." Otherwise, he was in good health, except that he had always been of a "nervous disposition" and quick-tempered. Nothing in his past history was suggestive of heart disease nor of any rheumatic manifestation.

There were no abnormal signs elicited on physical examination. Fluoroscopy of the chest indicated no enlargement of any of the cardiac chambers. The Kahn test was positive, and he received an intensive course of penicillin

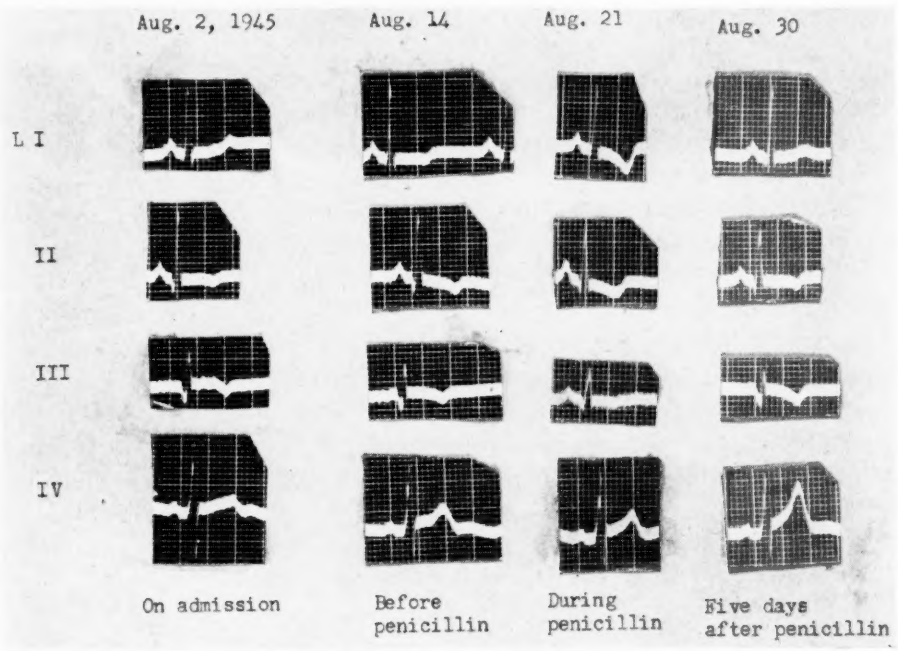

Fig. 7 (Patient 25).—Note changes in T_1 from upright to inversion and return to normal within a month. Changes during penicillin therapy considered coincidental.

Fig. 8 (Patient 17).—P-R interval, 0.28 second.

therapy (2,400,000 units in a seven-day period). The sedimentation rate was elevated (33 mm. in an hour) on admission and was 22 mm. two months later. During the last month of his hospitalization it was normal (10 mm. or less).

The outstanding feature in the electrocardiograms was the inversion of T_2 and T_3 (Fig. 7). This persisted throughout his course and was unaffected by exercise or the administration of ergotrate, 0.4 mg. intravenously, or full doses of atropine. An electrocardiogram taken during the period of penicillin treatment revealed an inversion of T_1 (Fig. 7). This disappeared after a

few weeks. The significance of this finding and the possible relationship of the electrocardiographic pattern, as well as the elevated sedimentation rate to his syphilis and the penicillin employed in its treatment, are difficult to evaluate. It is extremely questionable that the penicillin played any part in the production of the inverted T_1

It must be remembered that during the entire period the patient was ambulatory, although the symptomatology was the same and the examination was not productive of abnormal findings. The course in the hospital was otherwise uneventful, and on discharge after three months the symptoms continued unabated. It was finally concluded that the subject did not have organic heart disease but that his symptoms were functional in origin.

SUMMARY AND CONCLUSIONS

1. Five thousand, five hundred and twenty electrocardiographic studies made on 4,810 patients at a large Army regional hospital revealed fifty-one, or 1.0 per cent, of this total with abnormal T waves despite the absence of other criteria of heart disease. In twenty-three the bizarre T waves were confined to the chest leads and in twenty-eight appeared in the limb leads.

2. The subjects studied were of both sexes ranging in age from 19 to 47 years, the average being 29.9 years.

3. Constitutional types varied widely between the sthenic and asthenic; the majority (twenty-six, or 65 per cent) were of medium build.

4. A majority of the subjects in the series (thirty-one, or 6 per cent) presented definite psychoneurotic tendencies. However, thirteen had ailments unrelated to heart disease, and in seven no evidence of illness of any kind was determined.

5. In forty-three out of fifty patients (86 per cent of the total) standard exercise was successful in converting the bizarre T waves to normal.

6. The underlying theoretical considerations for the abnormal T waves were discussed. The possibility that at least in some instances these were merely physiologic variants was ventured. Many were undoubtedly based on autonomic nervous system imbalance.

7. One should be aware of a "normal" inversion of the T wave in order to avoid a mistaken diagnosis of heart disease.

I wish to express my appreciation for the technical assistance rendered by Lieutenant Gladys Eklund, Army Nurse Corps, and Corporal Ted Weible.

REFERENCES

1. Wilson, F. N., and Finch, R.: The Effect of Drinking Iced-Water Upon the Form of the T Deflection of the Electrocardiogram, Heart **10**: 275, 1937.
2. Lewis, T., and Gilder, M. D.: The Human Electrocardiogram; A Preliminary Investigation of Young Male Adults, to Form a Basis for Pathological Study, Phil. Tr. London, s.B. **102**: 351, 1913.
3. Ferguson, D., and O'Connell, J. T.: Cardiovascular Observations, U. S. Nav. M. Bull. **24**: 860, 1926.
4. Hall, G. E., Steward, C. B., and Manning, G. W.: The Electrocardiographic Records of 2,000 R.C.A.F. Aircrew, Canad. M. A. J. **46**: 226, 1942.

5. Graybiel, A., McFarland, R. A., Gates, D. C., and Webster, F. A.: Analysis of the Electrocardiograms Obtained From 1,000 Young Healthy Aviators, Am. Heart J. **27**: 524, 1944.
6. Scherf, D., and Weissberg, J.: The Alterations of the T Waves Caused by a Change of Posture, Am. J. M. Sc. **201**: 693, 1941.
7. Sigler, L. H.: Electrocardiographic Change Occurring With Alterations of Posture From Recumbent to Standing Positions, Am. Heart J. **15**: 146, 1938.
8. White, P. D., Chamberlain, F. L., and Graybiel, A.: Inversion of the T Waves in Lead II Caused by a Variation in Position of the Heart, Brit. Heart J. **3**: 233, 1941.
9. Wendkos, M. H.: The Influence of Autonomic Imbalance on the Human Electrocardiogram. I. Unstable T waves in the Precordial Leads From Emotionally Unstable Persons Without Organic Heart Disease, Am. Heart J. **28**: 549, 1944.
10. Graybiel, A., and White, P. D.: Inversion of the T Wave in Lead I or II of the Electrocardiogram in Young Individuals With Neurocirculatory Asthenia, With Thyrotoxicosis, in Relation to Certain Infections, and Following Paroxysmal Ventricular Tachycardia, Am. Heart J. **10**: 345, 1935.
11. Maizner, F., and Krause, M.: The Influence of Fear on the Electrocardiogram, Brit. Heart J. **2**: 221, 1940.
12. Dupuy, Homer: Normal Variations of the T Wave Seen Among Soldiers, New Orleans M. & S. J. **96**: 239, 1943.

OBSERVATIONS ON THE EFFECT OF THEOPHYLLINE AMINOISOBUTANOL IN EXPERIMENTAL HEART FAILURE

JOHN R. SMITH, M.D., AND JULIUS JENSEN, M.D.
ST. LOUIS, MO.

XANTHINE drugs, particularly theophylline preparations, have had wide clinical use in cardiac disease because of their diuretic effect, their beneficial action in cardiac dyspnea, and their coronary dilator properties. The xanthine drugs also stimulate the myocardium directly,[1-3] although this action usually has not been stressed as of clinical importance.[4, 5]

In a previous report Steinberg and Jensen[6] described their study of the effect of theophylline aminoisobutanol in patients with congestive heart failure. They found that administration of the drug in such patients usually lowered the venous pressure and resulted in shortening of the circulation time. The diminution of venous pressure was striking when it was initially elevated, but it was nevertheless often further diminished even when normal. The observations suggested that these beneficial actions result from: (1) direct myocardial stimulation by the drug; (2) improved myocardial function resulting from increased coronary flow; (3) action of the drug to produce peripheral vasodilatation; or (4) a combination of these actions. The results of Steinberg and Jensen were so striking that it was decided to investigate certain pharmacologic actions of theophylline aminoisobutanol in experimental heart failure, with the hope of clarifying the mechanism of the clinical effects noted by them.

METHOD

In these experiments, where heart failure was to be produced and then studied with respect to drug effect, it was desirable to record all features of myocardial performance as accurately as possible. The heart-lung preparation was chosen as the best means of observing these factors. In Fig. 1 is illustrated the experimental setup in principle.

Heart-lung preparations were made in the usual way and then removed from the body of the dog. Tensions within the right and left auricles were measured by simple manometers connected to the superior vena cava and through a cannula inserted into one of the pulmonary veins, respectively. Cardiac output was continuously measured by a siphon recorder. A rough estimate of cardiac size was obtained by means of a graduated pair of outside calipers stitched to the epicardium. Inflow of blood into the left circumflex coronary artery was recorded by the differential pressure orifice meter devised by Gregg

From the Department of Internal Medicine and the Oscar Johnson Institute for Medical Research, Washington University School of Medicine.

This work was supported by a grant from the Wm. S. Merrell Co., Cincinnati, Ohio.

Presented in part before the Central Society for Clinical Research, Chicago, Ill., Nov. 2 and 3, 1945.

Received for publication, April 8, 1946.

and Green,[7] the light beam from which was projected into an electrocardiographic photokymograph. Standardization of the instrument and quantitative interpretation of inflow curves have been described[7] and were carefully followed in these experiments. Arterial blood pressure was obtained using a Wiggers optical manometer; the light beam from this manometer also was projected into the kymograph and was recorded simultaneously with the coronary inflow curve on a bromide film.

When control observations had been made, heart failure was produced by the careful administration of 20 per cent chloral hydrate (1 c.c. every three minutes) to the perfusing blood. Approximately 0.8 Gm. of chloral hydrate was usually sufficient to produce definite heart failure without causing irretrievable cardiac damage.[8] With heart failure thus established, theophylline

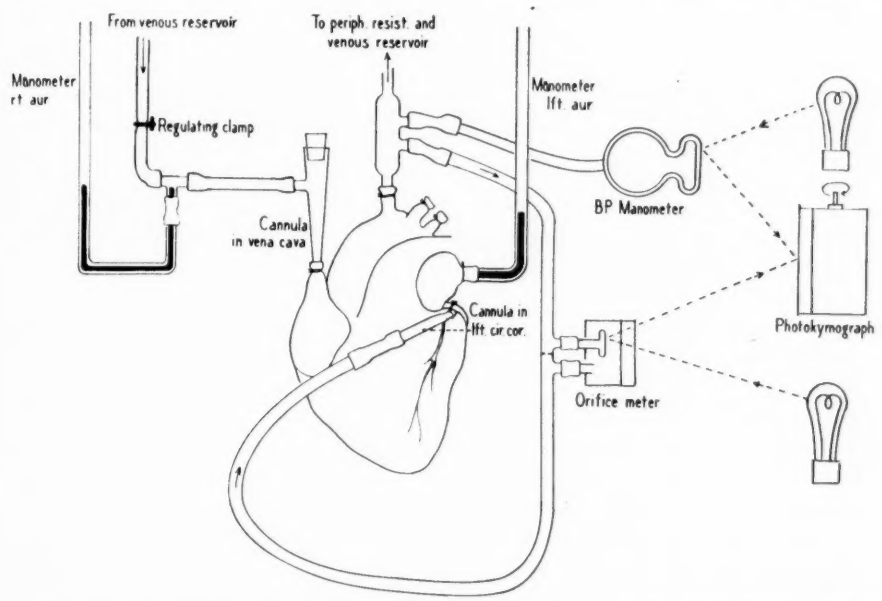

Fig. 1.—Diagrammatic sketch of heart-lung preparation and the instruments applied to it for the evaluation of myocardial function. Simple manometers are connected into the tube from the venous reservoir and into the left auricle. The flowmeter records inflow into the left circumflex coronary artery. Calipers used to estimate cardiac size and siphon for constant recording of cardiac output are not shown.

aminoisobutanol* (0.06 Gm.) was injected into the perfusing blood. The effects of the drug were observed until cardiac function became "stabilized." These experiments were performed on fourteen heart-lung preparations with consistent findings throughout.

As the study developed, it became desirable to ascertain changes of blood flow through edematous lungs in the course of heart failure and to note the effect of theophylline aminoisobutanol on pulmonary edema and congestion. In these observations the modified heart-lung preparation of Dusser de Barenne[9] was employed. In such a preparation blood is carried from the venous reservoir

*Wm. S. Merrell Co.

directly to the pulmonary artery. The left ventricle performs its normal complement of work; the right ventricle performs no work but serves as a receptacle for almost the whole of the coronary venous blood (see Fig. 2). At any time the arterial blood may be shunted around the artificial peripheral resistance and collected, together with coronary venous blood, in a recording siphon. These combined flows, therefore, represent total pulmonary flow. Heart failure was produced by using 20 per cent chloral hydrate as in the foregoing experiments, except that sufficiently more chloral hydrate was given to bring the heart to a standstill. Intense pulmonary edema and congestion occurred. The rate of blood flow through the lungs was then determined. Theophylline aminoisobutanol (0.06 Gm.) was added to the perfusate as the rate of blood flow through the lungs was continuously observed. Such observations were carried out on four de Barenne preparations.

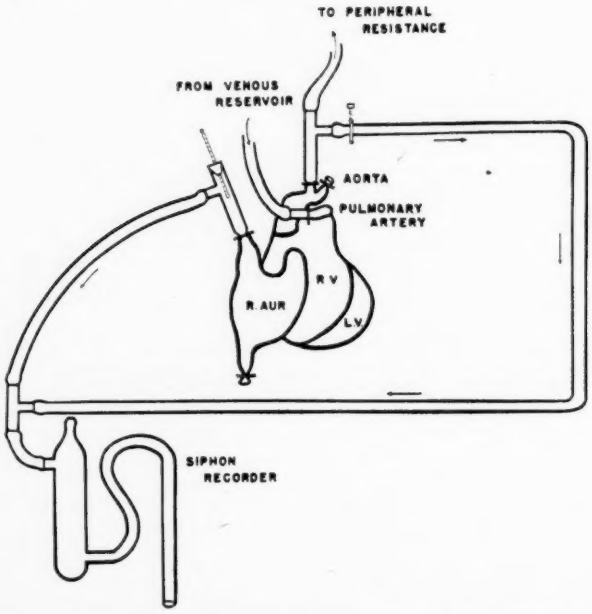

Fig. 2.—Schematic diagram of the modified heart-lung preparation of Dusser de Barenne (lungs are not shown). Blood from the venous reservoir is carried directly to the pulmonary artery, passes through the lungs, and is pumped through a peripheral resistance by the left ventricle. The right ventricle does no work. A cannula in the superior vena cava collects a large part of the coronary venous blood. The stream to the peripheral resistance may be clamped at will, and blood from the left ventricle may be shunted to the siphon-recorder and collected, together with coronary venous blood. The combined flows represent total pulmonary flow (cf. text).

RESULTS

Production of Heart Failure in the Heart-Lung Preparation.—When chloral hydrate had been added to the blood in the heart-lung, failure of the myocardium was manifested in the following series of events (see Table I): (1) Dilatation of the heart occurred, as shown by an increase in the diameter of the ventricles. Simultaneously, a rise of pressure within the left auricle was observed over a period of from fifteen to thirty minutes to approximately 10 to 11 cm. of water.

TABLE I. REPRESENTATIVE PROTOCOL OF EXPERIMENTAL HEART FAILURE IN HEART-LUNG PREPARATION AND THE EFFECT OF THEOPHYLLINE AMINOISOBUTANOL

EVENT	CARDIAC OUTPUT (C.C. PER MIN.)	BLOOD PRESSURE	RIGHT AURICLE (CM. OF H_2O)	LEFT AURICLE (CM. OF H_2O)	VENTRICULAR DIAMETER (CM.)	RATE	CORONARY INFLOW CYCLE (C.C.)	CORONARY INFLOW (C.C. PER MIN.)	REMARKS
Control	630	95/60	4.4	4.9	5.5	166	0.23	38.1	
Chloral hydrate (0.8 Gm. 12 m)	—	—	—	—	—	—	—	—	
Heart failing	480	90/60	6.6	11.0	6.7	111	0.35	38.8	Pulmonary edema and congestion
Theophylline (3 min.)	600	100/60	5.5	6.6	5.5	115	0.32	36.8	Myocardial stimulation; pulmonary edema subsides

Usually, right intra-auricular tension was not materially altered unless terminal severe myocardial failure supervened. Heart failure produced in this way was manifested, therefore, predominantly as left ventricular failure. (2) With dilatation of the heart a fall in cardiac output (from 25 to 40 per cent) occurred. The blood pressure diminished. The heart rate decreased. (3) Coronary arterial inflow *increased* during the course of cardiac failure exemplified by increase in inflow during each cardiac cycle. The decrease in heart rate usually rendered the net inflow per minute essentially unchanged. (4) The lungs showed intense congestion and edema with emphysema, parenchymal hemorrhage, and fluid in the tracheal cannula. Heart failure was not permitted to become more severe, as irremediable failure than occurred with loss of the experiment.

Effect of Theophylline Aminoisobutanol Upon Heart Failure.—The course of events following the administration of theophylline aminoisobutanol may be followed in Table I. Without exception the first measurable effect of the drug (occurring within one minute) was the rapid clearing of pulmonary edema and congestion. The run-off from the lungs appeared to increase so quickly that, momentarily, the left intra-auricular tension was driven up an additional 6 to 10 cm. of water, and transient further dilatation of the heart occurred. Thereafter, an obvious marked stimulation of myocardial action was observed. The heart beat vigorously. Within a few beats dilatation of the heart was completely removed. Cardiac output increased. Within three minutes the heart had resumed its "normal" state, and the entire preparation appeared to function as effectively as in the control period. Coronary arterial inflow was not essentially changed during the period of recovery from heart failure. Inflow during each cardiac cycle tended to decrease, and net inflow remained essentially unaltered with the rise in heart rate.

Effect of Theophylline Aminoisobutanol on Pulmonary Edema and Congestion in the de Barenne Preparation.—In the foregoing experiments the administration of theophylline appeared to cause a remarkably rapid clearing of pulmonary edema. In this series of experiments pulmonary blood flow was greatly

TABLE II. RECORD OF TWO EXPERIMENTS USING DE BARENNE PREPARATION*

PULMONARY FLOW—CONTROL (C.C. PER MIN.)	LUNG CONGESTED—HEART STOPPED (C.C. PER MIN.)	AFTER THEOPHYLLINE NO HEART BEAT (C.C. PER MIN.)
321	191	256
400	300	360

*Blood flow through the lungs before and during pulmonary congestion and edema produced by myocardial failure. The effect of theophylline aminoisobutanol on blood flow through the lungs when congested and edematous.

reduced during the severe pulmonary congestion and edema resulting from myocardial failure and standstill (see Table II). With the addition of theophylline (0.06 Gm.), there was an immediate increase in blood flow through the lungs with prompt subsidence of congestion and edema. By this means pulmonary flow was restored to approximate control values.

DISCUSION

From the experimental data at hand, the most striking—and probably the most important—effect of theophylline aminoisobutanol in heart failure is its action upon the myocardium. In all of the experiments the drug quickly induced marked, obvious vigor of ventricular contraction and prompt removal of cardiac dilatation. This phenomenon had been noted by other investigators[1-3] upon normally beating experimental hearts, and it was attributed by Heathcote[1] to its action directly upon the heart muscle. The question was then raised as to whether improvement in myocardial function resulted from coronary vasodilatation produced by the drug, with a corresponding increase in myocardial blood supply. That the xanthine drugs provoke coronary dilatation has been amply shown.[10-12] However, in these experiments an increase of coronary inflow (in each cardiac cycle) occurred in the course of failing integrity of the myocardium, despite a falling cardiac output and blood pressure and a rising tension in the left auricle. Since net coronary arterial inflow appears to be dependent in great part upon cardiac output[13] in normally beating experimental hearts, the increased inflow noted here is possibly due to peripheral coronary dilatation. These findings are in agreement with the experience of others. Gregg and Green[14] observed marked increase in coronary flow (under constant perfusion pressure) following transient ischemia of the heart muscle; more recently Green and Wegria[15] demonstrated an increase in myocardial irrigation during anoxia of the myocardium. It seems probable, therefore, that the effect of theophylline upon the coronary vascular bed in heart failure, which is already dilated, is largely annulled and that the drug exerts its beneficial action by direct stimulation of myocardial contractions. Visscher[16] has pointed out that in myocardial decompensation rising pressures in the right auricle and ventricle may impede the escape of blood from the coronary venous system to curtail coronary flow generally. In our preparations there was no significant rise of right intra-auricular tension. Therefore, it cannot be stated from our data whether impedance of coronary flow may have occurred had the circulation been intact, or what the effect of theophylline may be under such circumstances. The latter consideration is a recognized disadvantage of the heart-lung preparation.

In the light of available experimental evidence the mechanism of the removal of pulmonary edema by theophylline is difficult to evaluate. Friedberg, Katz and Steinitz[17] found in trained, unanesthetized dogs that the administration of aminophylline led to a mild, sustained elevation of mean pulmonary arterial tension. In their opinion the beneficial action of theophylline clinically could not be explained by the relief of congestion. However, they further suggested that theophylline might have an opposite effect when pulmonary congestion is present. On the other hand, Bock[18] observed that the xanthines increased blood flow through isolated lungs. Our observations suggest that marked vasodilatation occurs when theophylline is administered in pulmonary congestion. Blood impounded in the lungs escapes, and fluid rapidly leaves the alveolar air spaces to re-enter the circulation. Whether other factors, such as capillary permeability, are altered by the drug directly cannot be stated at present. In the heart-lung preparation the additional factors of improved myocardial function and release of increased pulmonary venous tension are also undoubtedly important in the removal of pulmonary edema.

Experimental evidence further indicates that theophylline preparations produce peripheral vasodilatation. Many years ago Phillips and Bradford[19] and Beco and Plumier[20] demonstrated increased blood flow through the renal vessels and other parts of the vascular bed by xanthine drugs. Sollman and Pilcher[21] and Phillips and Bradford[19] showed, by oncometric methods, that these drugs increased the size of the spleen in experimental animals. From the clinical standpoint, therefore, it seems probable that in addition to enhancing myocardial function and improving pulmonary blood flow theophylline also dilates, to some extent, the peripheral vascular bed. These factors, taken together, may improve the failing heart and circulation.

CONCLUSIONS

1. Heart failure was produced in the heart-lung preparation using chloral hydrate as a myocardial intoxicant. The administration of theophylline aminoisobutanol provoked striking stimulation of myocardial contraction with prompt restoration of effective function of the heart musele.

2. Pulmonary edema and congestion, in the course of failure in the heart-lung preparation, appeared to be removed promptly by theophylline aminoisobutanol. This phenomenon was further examined in the modified de Barenne heart-lung preparation. In was found that pulmonary blood flow was greatly curtailed in pulmonary congestion from heart failure. The addition of theophylline aminoisobutanol caused a rapid removal of pulmonary edema and congestion with restoration of free blood flow through the lungs.

3. We find no evidence from these observations that improvement in the failing experimental heart is dependent upon dilatation of the coronary arterial system by theophylline.

4. It is suggested that clinical benefit with theophylline aminoisobutanol in patients with heart failure results from improved myocardial function and pulmonary flow. Peripheral vasodilatation induced by the drug may also further benefit the disturbed circulation in such patients.

REFERENCES

1. Heathcote, R. St. A.: The Action of Caffeine, Theobromine and Theophylline on the Mammalian and Batrachian Heart, J. Pharmacol. & Exper. Therap. **16:** 327, 1921.
2. Bodo, R.: The Effect of "Heart-Tonics" and Other Drugs Upon the Heart-Tone and Coronary Circulation, J. Physiol. **64:** 365, 1927-28.
3. Bock, J.: Ueber die Wirkung des Coffeins und des Theobromins auf das Herz, Arch. f. exper. Path. u. Pharmakol. **43:** 367, 1900.
4. Nathanson, M. H., and Fitzgibbon, J. P.: Pharmacology of Cheyne-Stokes Respiration, Am. Heart J. **17:** 691, 1939.
   Greene, J. A., Paul, W. D., and Feller, A. E.: The Action of Theophylline With Ethylenediamine, J. A. M. A. **109:** 1712, 1937.
5. White, P. D.: Heart Disease, New York, 1944, The Macmillan Company.
6. Steinberg, F., and Jensen, J.: The Effect of Theophylline Aminoisobutanol on the Circulation in Congestive Heart Failure, J. Lab. & Clin. Med. **31:** 857, 1946.
7. Gregg, D. E., and Green, H. D.: Registration and Interpretation of Normal Phasic Inflow Into a Left Coronary Artery by an Improved Differential Manometric Method, Am. J. Physiol. **130:** 114, 1940.
8. Fahr, G., and Buehler, M. S.: A Physiologic Definition of Acute Congestive Heart Muscle Failure, Am. Heart J. **25:** 211, 1943.
9. Dusser de Barenne, J. G.: Ueber eine Methode zur genauen Bestimmung des gesamten Koronarkreislaufs, Pflüger's Arch. f. d. ges. Physiol. **188:** 281, 1921.
10. Fisher, I., Guggenheimer, H., and Müller, E. A.: Ueber die Beeinflussung von Koronardurchblutung und Herztonus durch Theophyllinpräparate und Strophanthin nach Untersuchungen am Starlingschen Herz-Lungenpräparat, Deutsche med. Wchnschr. **54:** 1584, 1928.
11. Lindner, E., and Katz, L. N.: Further Observations on the Action of Drugs on the Calibre of Coronary Vessels, J. Pharmacol. & Exper. Therap. **72:** 306, 1941.
12. Boyer, N. H., and Green, H. D.: The Effect of Nitrites and Xanthines on Coronary Inflow and Blood Pressure in Anesthetized Dogs, Am. Heart J. **21:** 199, 1941.
13. Katz, L. N., Wise, W., and Jochim, K.: Factors Controlling Conorary Circulation, Proc. Central Soc. Clin. Research **17:** 22, 1944; also J. Lab. & Clin. Med. 30: 374, 1945.
14. Gregg, D. E., and Green, H. D.: Effects of Viscosity, Ischemia, Cardiac Output and Aortic Pressure on Coronary Blood Flow Measured Under a Constant Perfusion Pressure, Am. J. Physiol. **130:** 108, 1940.
15. Green, H. D., and Wegria, R.: Effects of Asphyxia, Anoxia and Myocardial Ischemia on the Coronary Blood Flow, Am. J. Physiol. **135:** 271, 1942.
16. Visscher, M. B.: The Restriction of the Coronary Flow as a General Factor in Heart Failure, J. A. M. A. **113:** 987, 1939.
17. Friedberg, L., Katz, L. N., and Steinitz, F. S.: The Effect of Drugs on the Pulmonary and Systemic Arterial Pressures in the Trained Unanesthetized Dog, J. Pharmacol. & Exper. Therap. **77:** 80, 1943.
18. Bock, H. E.: Zur Gefässwirkung des Strophanthins, Theozins und Coffeins. Untersuchungen an der überlebenden Extremität und an der über lebenden Lunge. Arch. f. exper. Path. u. Pharmakol. **166:** 634, 1932.
19. Phillips, C. D. F., and Bradford, J. R.: On the Action of Certain Drugs on the Circulation and Secretion of the Kidney, J. Physiol. **8:** 117, 1887.
20. Beco, L., and Plumier, L.: Action cardio-vasculaire de quelques dérivés xanthiques, J. de physiol. et de path. gén. **8:** 10, 1906.
21. Sollman, T., and Pilcher, J. D.: The Actions of Caffeine on the Mammalian Circulation, J. Pharmacol. & Exper. Therap. **3:** 17, 1912.

THE EFFECT OF THEOPHYLLINE AMINOISOBUTANOL ON THE CIRCULATION IN CONGESTIVE HEART FAILURE

FRANZ U. STEINBERG, M.D., AND JULIUS JENSEN, M.D.
ST. LOUIS, MO.

INTRODUCTION

IT IS generally accepted that theophylline has important but still incompletely known effects on the cardiovascular system.[1, 2] This paper is concerned with some of these effects in the presence of congestive heart failure. We were first impressed with the decrease in venous pressure which we observed following the administration of theophylline aminoisobutanol, especially when the venous pressure was increased. This effect has been reported in the literature but has not been thoroughly investigated. Greene, Paul, and Feller[3] had noted it when studying the effect of theophylline upon the mechanism of dyspnea. Robertson and Faust[4] recommended the use of theophylline for lowering the venous pressure in congestive heart failure, but they employed doses too small to obtain significant effects. We could find no evidence that the subject had been further investigated. We also noted that the arm-to-tongue circulation time was shortened after administration of this drug, a finding not previously reported in the literature. A clinical study was therefore undertaken in order to investigate further these changes in venous pressure and circulation rate which follow the administration of theophylline.

METHOD

Theophylline aminoisobutanol was given intravenously in doses of 0.24 and 0.48 Gm. For comparison, theophylline with ethylenediamine (aminophylline) was used in a smaller number of cases. A few tests were made with the sublingual administration of 0.6 mg. of glyceryl trinitrate (nitroglycerin).

The venous pressures were determined by a modification of the method of Moritz and Tabora.[5] The apparatus used is shown in Fig. 1. The precautions recommended by Lyons, Kennedy, and Burwell[6] were closely observed. The needle was left in the vein during the entire period of observation. Clotting was prevented by adding a 2.5 per cent solution of sodium citrate at the rate of from 6 to 8 drops per minute. Theophylline and the drugs used for the determination of the circulation time were injected through a three-way stopcock. Therefore with one venepuncture it was possible to follow the venous pressure for one or two hours, introduce the drugs to be tested, and determine the circulation time as often as desired. The spontaneous changes of the venous pressure were studied by this method for varying periods of time, generally about twenty minutes, before the introduction of any drug. After a drug had

From the Department of Medicine, Washington University School of Medicine, and the St. Louis City Hospital, Max C. Starkloff Memorial.

This study was supported by a grant from the Wm. S. Merrell Company, Cincinnati, Ohio.
Received for publication, April 8, 1946.

been administered readings were taken every few minutes until the effect had worn off or the test had to be interrupted for other reasons, especially restlessness of the patient.

The arm-to-tongue circulation time was determined before and one or more times after the drug had been administered. For most tests decholin was used. When this was not available, from 2 to 3 c.c. of a 25 per cent solution of magnesium sulfate or 2 c.c. of a saturated solution of saccharin were used instead. Saccharin was found less suitable as it may cause a painful spasm of the vein into which it is injected. This gives rise to a disturbing temporary elevation of the venous pressure.

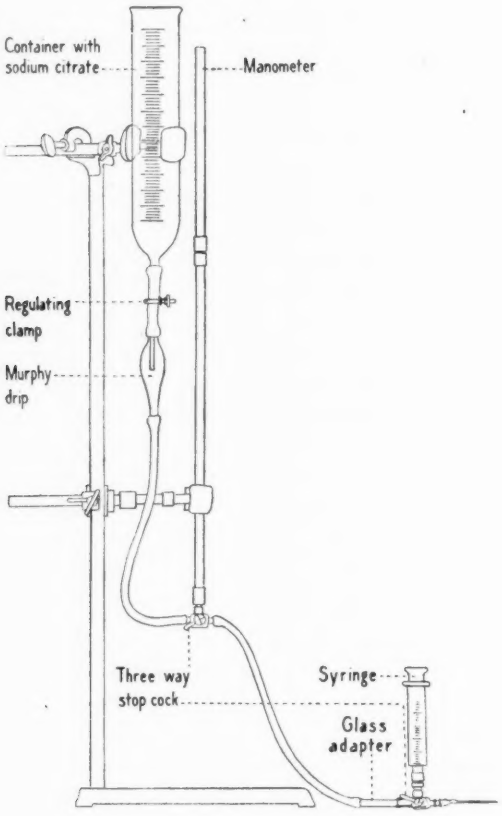

Fig. 1.

Plasma volume determinations* were carried out according to the method of Gibson and Evans[7] as modified for the photoelectric colorimeter by Gibson and Evelyn.[8] Gibson and Evans found that after the injection of from 15 to 20 mg. of Evans blue the dye was completely mixed with the blood in from ten to twenty minutes. However, in our cases of congestive heart failure the

*We are greatly indebted to Dr. R. Dubach, for her assistance in carrying out these determinations.

circulation was retarded and from forty-five to sixty minutes were allowed to elapse. After this period three blood specimens were drawn at from seven- to ten-minute intervals. Then 0.48 Gm. of theophylline aminoisobutanol was injected, and from five to ten minutes thereafter three more blood specimens were drawn. The dye concentrations were determined by means of the photoelectric colorimeter, and the plasma volume was computed following directions by Gibson and Evelyn. Venous pressure determinations were made simultaneously.

RESULTS

Preliminary Observations.—In fourteen patients with congestive heart failure, heart rate, arterial blood pressure, venous pressure, arm-to-tongue circulation time, and vital capacity were determined before and fifteen minutes after the intravenous administration of 0.24 or 0.48 Gm. of theophylline aminoisobutanol In almost all cases the administration of the drug was followed by a decrease of venous pressure and shortening of the circulation time. Consequently it was decided to investigate further these two effects. Heart rate, arterial pressure, and vital capacity remained essentially unchanged.

Venous Pressure

Changes of Venous Pressure Apart From Drug Effect.—It soon became apparent that the venous pressure varied widely apart from any drug effect. Frequently the highest reading was obtained immediately after the needle had been introduced into the vein. Within a few minutes the pressure fell to a lower level where it remained stationary. Pogany[9] states that the mechanical irritation of venepuncture may give rise to a spastic contraction of the vein with a transient elevation of venous pressure. This phenomenon was not observed in all patients, but it could usually be repeated in the same individual. Relaxation or sleep caused a spontaneous fall in venous pressure. Coughing, vomiting, or other acts associated with an elevation of the intrathoracic pressure

TABLE I

FALL IN VENOUS PRESSURE (MM. OF WATER)	CONTROL GROUP (NO. OF CASES)	THEOPHYLLINE AMINOISO-BUTANOL GROUP (NO. OF CASES)	AMINOPHYLLINE GROUP (NO. OF CASES)	NITROGLYCERIN GROUP (NO. OF CASES)
1 to 19	35	7	4	1
20 to 39	24	16	4	5
40 to 59	3	9	8	4
60 to 79	3	6	2	2
80 to 99	0	1	2	1
100 to 119	0	0	1	0
120 to 139	2	0	0	0
Total number	67	39	21	13
Range	2 to 128	8 to 77	11 to 118	18 to 88
Mean	23.6	36.7	48.0	47.8
Standard deviation	22.3	16.5	27.8	20.2
Median	18.0	33.0	48.0	39.0
Difference of means: Standard error		3.47	3.67	3.91

In the control group the fall of venous pressure was measured from the highest to the lowest reading obtained. When drugs were given it was measured from the reading just before the drug was administered to the lowest reading obtained within thirty minutes.

led to a steep rise of the venous pressure, followed by a rapid decline to the original level. The venous pressure usually rose during the apneic phase of Cheyne-Stokes respiration as previously reported by Meyer and Middleton.[10] Sometimes the pressure varied without apparent cause.

The following observations were made in sixty-seven control periods lasting from six to fifty-seven minutes, mostly about twenty minutes. The maximum variation from the highest to the lowest reading ranged from 2 to 128 mm. of water, with an average of 23.6 mm. of water (Table I).

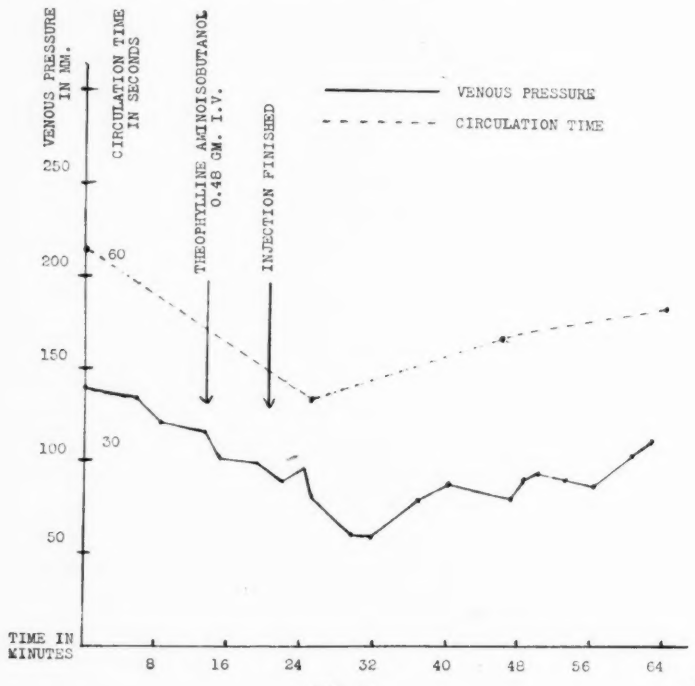

Fig. 2.

Theophylline Aminoisobutanol.—In the majority of cases the injection of theophylline aminoisobutanol caused an abrupt fall of the venous pressure. The drop usually began during the course of the injection which, for reasons of safety, was made slowly. Often the lowest point was reached with the end of the injection. In other cases the pressure continued to fall; it reached its low almost always within the first one-half hour. Thereafter the pressure fluctuated for some time in the vicinity of the lowest reading or it began to rise again. (Fig. 2.) Often changes attributed to the drug were obscured by changes due to other factors which sometimes caused rises before they could otherwise be expected.

To determine the extent of the decrease, the average of the last two readings before the drug was given was compared with the lowest reading obtained within thirty minutes. In thirty-nine cases in which 0.48 Gm. of theophyl-

line aminoisobutanol was given, the venous pressure decreased from 8 to 77 mm. of water with an average decrease of 36.7 mm. This is considerably more than the average maximum spontaneous variations of 23.6 mm. of water.

The distribution of the spontaneous decreases in venous pressure was compared with the distribution of decreases which followed the injection of theophylline aminoisobutanol and also with those which followed aminophylline and nitroglycerin (see below). The statistical analysis of these data is recorded in Table I. The difference between the means of the control group and the theophylline aminoisobutanol group is 3.47 times the standard error. This indicates that the differences are significant. This is notwithstanding the fact that the differences in the control series were the maximum ones obtainable, while the drug effect was measured consistently as the difference between the reading preceding the drug and the lowest reading obtained.*

The fall of the venous pressure was generally the more extensive the higher the pressure before injection. However, no quantitative correlation could be established between the height of the original venous pressure and the amount of decrease, expressed either in absolute figures or in percentages. This is not surprising since we were faced with a reaction which is subject to so many outside influences.

TABLE II

DRUG	REACTION ENDING WITHIN SIXTY MINUTES	REACTION LASTING MORE THAN SIXTY MINUTES	TEST TERMINATED BEFORE SIXTY MINUTES AND BEFORE END OF REACTION	TOTAL
Theophylline aminoisobutanol	19	4	15	38
Aminophylline	7	7	7	21
Nitroglycerin	7	2	4	13

The duration of the effect was often difficult to determine because it might be obscured by increases in venous pressure due to some of the factors previously mentioned. Sometimes restlessness necessitated premature termination of the test.

Arbitrarily the end of the reaction was thought to occur when three consecutive readings were within 10 per cent of the last reading before the drug was given. For comparison with the effect of other drugs, it was thought practical to divide the cases into those in which the effect ended within sixty minutes, those in which it persisted beyond sixty minutes, and those in which outside factors caused termination of the test within sixty minutes, yet before the reaction ended (Table II).

Aminophylline.—In twenty-one cases 0.48 Gm. of aminophylline was injected intravenously. The reaction followed the same pattern as described for theophylline aminoisobutanol. The decline of the venous pressure from the preinjection to the lowest reading ranged from 11 to 118 mm. of water with an aver-

*We are indebted to Dr. E. Gurney Clark, for his assistance in the statistical analysis of our data.

age of 48.0 mm. (Table I). The duration of the aminophylline effect on the venous pressure is seen in Table II. The impression that it lasts longer with this drug than with theophylline aminoisobutanol or nitroglycerin rests upon too few observations to be convincing.

Nitroglycerin.—In thirteen cases 0.6 mg. of nitroglycerin was given sublingually. Usually the pressure began to fall steeply within one minute after the administration of the drug. The decrease from the reading before nitroglycerin to the lowest value ranged from 18 to 88 mm. of water and averaged 47.8 mm. for the thirteen cases (Table I).

Failure of Response.—The cases in which the venous pressure failed to decrease fell into two groups. The larger group comprised cases in which the venous pressure was not elevated prior to drug administration. In these there was little opportunity for a significant decrease. The second group contained cases in which the venous pressure was definitely above normal but responded poorly to the drug, that is, fell to less than 20 mm. of water. We encountered five such cases in the theophylline aminoisobutanol group, two in the aminophylline, and none in the nitroglycerin group. The reason for the failure to react was uncertain, but most of the patients were markedly decompensated and failed to react to other therapeutic measures as well.

Repeated Tests.—In many cases the test was repeated once or several times in the same patient on different days in order to test the individual constancy of pattern. Again, the great spontaneous variations made comparison difficult. The venous pressure changed so much from day to day that rarely were the starting points the same.

Circulation Time

The arm-to-tongue circulation time was measured on 168 occasions. Being a "subjective" test it is influenced by the patient's ability to recognize the taste, but there was no doubt that theophylline aminoisobutanol often shortened the circulation time, more so the longer the original time. The second reading was taken approximately fifteen minutes after the beginning of the administration of the drug. The results are summarized in Table III.

TABLE III

DRUG	NUMBER OF CASES	DOSE	AVERAGE SHORTENING OF CIRCULATION TIME IN SECONDS
Theophylline aminoisobutanol	9	0.24 Gm.	10.4
Theophylline aminoisobutanol	20	0.48 Gm.	12.8
Aminophylline	9	0.48 Gm.	16.8
Nitroglycerin (sublingual)	9	0.6 mg.	8.6

There was relatively little difference between the effect of the various preparations. Perhaps nitroglycerin in the customary doses is somewhat less effective in accelerating the circulation. Not enough tests were made to determine how long the effect lasted, but we had the impression that it passed off in from thirty to ninety minutes or about simultaneously with the drop in venous pressure.

Comparison Between Circulation Time and Venous Pressure.—For the purpose of the following analysis the upper normal limits of the circulation time and venous pressure were considered to be twenty seconds and 120 mm., respectively. In our observations it was more common for the circulation time to be prolonged while the venous pressure was normal than for the reverse to occur. In only one case was the venous pressure elevated (158 mm.) while the circulation time was low (ten seconds); this patient had a marked anemia, a condition which accelerates the circulation. These observations would suggest that the slowing of the circulation time is a more fundamental change in congestive heart failure than is increase in venous pressure.

Following the drugs both venous pressure and circulation time were affected in most cases. Changes of less than five seconds in the circulation time or of less than 20 mm. for the venous pressure were not accepted as drug effects. In no case was the circulation time shortened without fall of the venous pressure; in eleven cases the venous pressure fell while the circulation rate remained unaffected. This observation again supports the impression that slowing of the circulation rate is more fundamental than increase of venous pressure in congestive heart failure. Four cases failed to respond in both respects.

Plasma Volume

Plasma volume determinations were carried out in six cases before and after the injection of 0.48 Gm. of theophylline aminoisobutanol. In no case

TABLE IV. DETERMINATION OF VENOUS PRESSURE AND PLASMA VOLUME BEFORE AND AFTER INJECTION OF THEOPHYLLINE AMINOISOBUTANOL

(PATIENT W. G.; AGE, 60 YEARS; DIAGNOSIS, ARTERIOSCLEROTIC HEART DISEASE)

TIME	VENOUS PRESSURE (MM. OF WATER)	PLASMA VOLUME (C.C.)
10:15	328	
10:21	288	3,873
10:23	274	
10:27	280	
10:29		3,750
10:30	264	
10:32	268	
10:37	272	
10:38	270	
10:39		3,894
10:40	268	
10:42	270	
10:42 to 10:46	Theophylline aminoisobutanol, 0.48 Gm. intravenously	
10:46	232	
10:47	224	
10:48	220	
10:49		3,894
10:51	226	
10:52	222	
10:54	222	
10:56	226	
10:57		3,894
10:59	230	
11:00	242	
11:02	254	
11:04	258	
11:05		3,923

could a significant change in dye concentration and plasma volume be observed, even though the venous pressure fell as usually. This indicates that there was no important shift of fluid from the blood into the tissues during the period of observation. A representative protocol of one of these tests is reproduced in Table IV.

DISCUSSION

Prolongation of the circulation time and elevation of the venous pressure are fundamental factors in the pathogenesis of congestive heart failure. An improvement in the circulation will necessarily be associated with a relief of venous congestion and an acceleration of the circulation. Our observations establish that theophylline produces these effects and that this drug, therefore, should have a definite place in the treatment of congestive heart failure. The effect begins almost instantaneously but soon wears off. Therefore, the drug will be most useful in emergencies. It can be used to precede digitalis action, which is slower in producing an effect but which is more sustained.

On the basis of clinical experience the use of theophylline has been recommended in the acute phases of congestive heart failure. However, the literature contains only a few references to its effect on the venous pressure. The reports by Greene, Paul, and Feller[3] and Robertson and Faust[4] have been mentioned previously. Wilkins, Haynes, and Weiss,[11] have reported the fall of venous pressure which follows the administration of nitrites.

Our observations give no explanation of the mechanism by which theophylline produces the observed reduction of venous pressure and circulation time. Several possibilities have to be considered: (1) peripheral vasodilation, (2) strengthening of the myocardium, (3) indirect improvement of myocardial efficiency due to an increase of the coronary circulation, and (4) a combination of several or all of these factors.

The fact that the xanthines dilate the small veins has been well established experimentally.[12-14] This effect would cause a sidetracking of part of the circulating blood, thus temporarily relieving the load on the failing heart. Such an effect could well account for a decrease of venous pressure and an acceleration of the circulation.

In the older literature there are found reports that the xanthines increase the capillary permeability for water, thereby causing a transudation of fluid from the blood into the tissues. This process would tend to reduce the plasma volume which is usually increased in congestive heart failure[15] and bring on the phenomena which we have observed. However, the reports concerning the increase of capillary permeability due to xanthines are based on inconclusive experimental evidence.[16] Our plasma volume studies indicate definitely that no appreciable amount of fluid leaves the blood stream concomitant with the fall of venous pressure. We were not concerned with the diuretic action which occurs later and may account for such changes as observed by Zak,[17] in which the time factor was not considered.

The question whether or not theophylline stimulates myocardial function and improves the coronary circulation could not be answered by our clinical studies.

SUMMARY

Theophylline aminoisobutanol by intravenous administration causes a fall in venous pressure and a shortening of the circulation time. These effects are the more pronounced the more these functions are elevated above normal.

The venous pressure begins to fall while the injection is still in progress. The effect persists for about sixty to ninety minutes. The circulation time apparently remains shortened for about the same period of time.

The fall in venous pressure is not accompanied by any changes in the plasma volume.

Aminophylline and nitroglycerin produce the same effects.

The venous pressure shows wide fluctuations apart from any drug effect.

REFERENCES

1. Council on Pharmacy and Chemistry: Limitations of Claims for Aminophylline and Other Xanthine Derivatives, J. A. M. A. **108:** 2203, 1937.
2. Starr, I., Gamble, C. J., Margolies, A., Donal, J. S., Jr., Joseph, N., and Eagle, E.: A Clinical Study of the Action of Ten Commonly Used Drugs on Cardiac Output, Work and Size; on Respiration, on Metabolic Rate and on the Electrocardiogram, J. Clin. Investigation **16:** 799, 1937.
3. Greene, J. A., Paul, W. D., and Feller, A. E.: The Action of Theophylline With Ethylenediamine, J. A. M. A. **109:** 1712, 1937.
4. Robertson, H. F., and Faust, F. B.: Theophylline With Isopropanolamine in Heart Disease, With Special Reference to Congestive Failure, J. Lab. & Clin. Med. **25:** 1066, 1940.
5. Moritz, F., and v. Tabora, D.: Ueber eine Methode beim Menschen den Druck in den oberflaechlichen Venen exakt zu bestimmen, Deutsches Arch. f. klin. Med. **98:** 475, 1910.
6. Lyons, R. H., Kennedy, J. A., and Burwell, C. S.: The Measurement of Venous Pressure by the Direct Method, Am. Heart J. **16:** 675, 1938.
7. Gibson, J. G., Jr., and Evans, W. A. Jr.: Clinical Studies of the Blood Volume. I. Clinical Application of the Method Employing Azo Dye "Evans Blue and Spectrophotometer, J. Clin. Investigation **16:** 301, 1937.
8. Gibson, J. G., Jr., and Evelyn, K. A.: Clinical Studies of the Blood Volume. IV. Adaptation of the Method to Photoelectric Microcolorimeter, J. Clin. Investigation **17:** 153, 1938.
9. Pogany, J.: Der Venendruck und seine klinische Bedeutung, Ergebn. d. inn. Med. u. Kinderh. **41:** 257, 1931.
10. Meyer, O. O., and Middleton, W. S.: The Influence of Respiration on Venous Pressure, J. Clin. Investigation **8:** 1, 1929.
11. Wilkins, R. W., Haynes, F. W., and Weiss, S.: The Rôle of the Venous System in Circulatory Collapse Induced by Sodium Nitrite, J. Clin. Investigation **16:** 85, 1937.
12. Sollmann, T.: A Manual of Pharmacology, ed. 6, Philadelphia, 1942, W. B. Saunders Company.
13. Sollmann, T., and Pilcher, J. D.: The Action of Caffeine on the Mammalian Circulation, J. Pharmacol. & Exper. Therap. **3:** 19, 1912.
14. Bock, H. E.: Zur Gefässwirkung des Strophanthins, Theozins und Coffeins, Arch. f. exper. Path. u. Pharmakol. **166:** 634, 1932.
15. Gibson, J. G., Jr., and Evans, W. A., Jr.: Clinical Studies of the Blood Volume. III. Changes in Blood Volume, Venous Pressure and Blood Velocity Rate in Chronic Congestive Heart Failure, J. Clin. Investigation **16:** 851, 1937.
16. Bauer, J., and Aschner, B.: Ueber Austauschvorgaenge zwischen Blut und Geweben, Deutsches Arch. f. klin. Med. **138:** 270, 1922.
17. Zak, E. R.: Tissue Action of Theophylline and Other Xanthines, Exper. Med. & Surg. **1:** 181, 1943.

STIMULATION OF THE CENTRAL NERVOUS SYSTEM BY CURARE (INTOCOSTRIN)

ROSELLEN E. COHNBERG
ST. LOUIS, MO.

THE increasing use of curare (Intocostrin)* as an adjunct to anesthesia and for other clinical purposes indicates the need for additional information regarding its action in the body. The work of West[1] seems to be the only recent extensive pharmacologic study of curare preparations. The investigation reported herein is a contribution to the toxicology of the particular curare alkaloid now readily available.

Intocostrin is reported by the manufacturers to owe its curare action (interference with the passage of impulses from motor nerves to skeletal muscles at the neuromuscular junction) almost entirely to its content of d-tubocurarine, an alkaloid found in Amazonian curare. This alkaloid has been crystallized and its chemical structure established fairly completely.[2] It is less potent than curarine, the characteristic alkaloid of curare from British Guiana.

METHODS

Intocostrin (from 0.5 to 10.0 units per kilogram)† was administered to rats, mice, guinea pigs, rabbits, and cats subcutaneously, intramuscularly, intraperitoneally, or intravenously. For all injection routes, the drug was diluted if necessary to keep the volume at least 0.4 c.c. The marginal ear veins of unanesthetized rabbits and the exposed femoral veins of rats were used for intravenous injections. The rats were lightly anesthetized with ether for the exposure of the veins and were allowed to recover to the point of moving spontaneously before the intocostrin was injected. Practically all the injections were made rapidly (within two to five seconds); a few slow (from one to four minutes) intravenous injections into rabbits indicated that the rate of injection did not make a significant difference in the effects. Crystalline d-tubocurarine (6.17 standard intocostrin units per milligram) was dissolved in Ringer's solution and administered in suitable dilution by rapid subcutaneous or intramuscular injection to mice and to a few rats and guinea pigs (from 0.5 to 1.0 mg. per kilogram).

Most of the experiments with intocostrin were done on rats (weighing from 150 to 200 grams). No difference in the susceptibility of the sexes was noted, but it was found that the amount of intocostrin required to kill the rats decreased as their stay in the laboratory was prolonged. The deciding factor in this effect was presumably either age or nutritition, but this was not determined. A homogeneous group of rats was used as far as possible for the sodium amytal series and its control (Table III).

Sodium amytal (from 75 to 100 mg. kilogram, intraperitoneally) was found to produce complete anesthesia in our rats without intocostrin. As the combination of this dose

From the Department of Pharmacology, Washington University School of Medicine.

Received for publication, May 4, 1946.

*E. R. Squibb & Sons, New Brunswick, N. J., kindly supplied the Intocostrin and crystalline d-tubocurarine chloride used in this investigation.

†Intocostrin is adjusted by the manufacturers to contain 20 units per cubic centimeter, a unit being measured by the rabbit head drop crossover method. In this method small increments of the drug are injected intravenously at fifteen-second intervals until the rabbit is unable to raise its head under reflex stimulus. An early description of the method is found in Bennett.[3], p. 1045 and Plate 34 We found that 1 unit per kilogram injected in two to ten seconds into the ear vein of each of two rabbits produced head drop that appeared in a few seconds and lasted a few minutes.

with intocostrin was usually fatal, sodium amytal was use at the minimum level (between 50 and 75 mg. per kilogram) that permitted the introduction of a hypodermic needle without causing the animal to withdraw its leg. Intocostrin (2 units per kilogram) was then injected subcutaneously or intramuscularly. In a few animals in the series, the sodium amytal was not injected until severe convulsions had developed following intocostrin injection.

Oxygen, a mixture of 5 per cent CO_2 and 95 per cent O_2, or cyclopropane, when used, was delivered at a rate of 1,000 c.c. per minute by an anesthesia gas machine into a 2 liter jar in which a rat was placed. Cyclopropane was started in the proportion of 3 parts to 7 parts of oxygen and then was reduced to the concentration required to maintain light anesthesia. Artificial respiration was administered manually or by breathing into a tube placed over the animal's nose.

Neostigmine methyl sulfate (from 0.5 to 0.75 c.c. 1/4000) was injected intramuscularly into two rats.

RESULTS

Effects of Intocostrin and d-Tubocurarine Chloride.—Preliminary injection of intocostrin into frogs confirmed the results of workers with other curare preparations by producing muscular relaxation when the injection was made into the lymph sac and stimulation and tonic convulsions when the injection was made into the cerebrospinal canal through the foramen magnum.

The mammals injected with either intocostrin or d-tubocurarine chloride manifested: (1) weakness, flaccidity, decrease of normal activity, paralysis (regarded as signs of curarization) and (2) with the same or a larger dose, tics, tremors, convulsions (signs of nervous stimulation). All species showed both stimulation and curarization but to varying degrees; muscular relaxation was more prominent in rabbits and cats, nervous stimulation in rats and mice. The course of the poisoning following subcutaneous or intramuscular injection in rats will be described, and then the variations observed with different species or modes of injection will be pointed out. The results of all of these experiments are summarized in Table I.

Small subcutaneous or intramuscular doses in rats (from 0.5 to 0.75 units per kilogram) produced weakness, trembling, and marked decrease of spontaneous movements but hyperreactivity to external stimuli. Rats given 1 unit per kilogram exhibited gnashing of teeth, facial tics, twitching of back muscles, and spasms of respiratory muscles. The animals recovered from these subconvulsive doses in varying short lengths of time and showed no apparent after-effects.

Generalized convulsions of clonic-epileptiform type were produced in all rats given 1.25 or more units per kilogram. The convulsions were preceded by incoordination in walking, generalized trembling and weakness, decreased spontaneous activity, and the other effects observed with smaller doses. A characteristic convulsion began at the mouth, travelled up to the ears and eyes, and thence, like a shudder, down the back; the tail was drawn slightly dorsally. The accompanying or succeeding violent clonic movement of the whole body resulted in some animals in leaps of five or six inches upward and from one to twelve inches forward. The convulsions seemed to arise spontaneously or in response to almost any external stimulus, no matter how slight. An individual

convulsive seizure was occasionally introduced by a tonic phase. Each seizure lasted from a few seconds to a minute. The generalized convulsions were succeeded by a stage of localized muscle spasms and ties. The ability to move in response to external stimuli was not lost at any time in nonfatal cases, nor in 10 per cent of the fatal cases until the very end. In most of the fatal cases, it was retained through the first violent convulsions but disappeared in the asphyxial stage.

The time-course of the poisoning with convulsive subcutaneous or intramuscular doses in rats was as follows: No matter how large the dose, convulsions seldom started within thirty seconds after the injection. The average time to the first convulsion was about three minutes, whether the animal was left free to run about or was confined to a small area. Trembling began about one minute before the first convulsion. Individual convulsions were no more violent in the fatal than in the nonfatal cases, but they were more numerous; the stage of generalized convulsive seizures lasted on the average six minutes. With a nonfatal dose convulsions were followed by recovery in forty-five minutes to two hours. With a fatal dose respiration ceased on the average fifteen and one-half minutes after the injection, and the heart beat was not palpable two minutes later unless artificial respiration was instituted.

Respiration appeared to be normal in rate, depth, and character until the onset of convulsions or even during mild convulsions. With the appearance of violent and frequent convulsions (that is, three seizures per minute), apneusis occurred during the convulsions. Cyanosis was not visible around the mouth or in the paws until this stage was reached, but then it soon became marked, in spite of normal or deep-labored breathing between convulsions. Typical asphyxial gasps eventually developed, and in fatal cases death was due to asphyxia.

The subcutaneous or intramuscular dose required to kill 95 per cent of the rats was 2 units per kilogram. It appeared to be the same whether the drug was given all in one injection or in fractional injections following each other within a few minutes. Although rapidly repeated doses were markedly cumulative, rats given a convulsive, subfatal dose on three successive days seemed entirely normal as to gait, appetite, motor activity, nervous system reactivity, and escape patterns.

The effects of a given dosage of intocostrin were apparently the same after intraperitoneal injection (seven rats) as after subcutaneous or intramuscular injection. With intravenous injection (twenty-six rats), however, the dosage required for any given effect was smaller (Table I), and the effects appeared more promptly. Apneustic breathing began almost immediately after intravenous injection, and there was time for only a few ties and a few clonic movements before death occurred (sometimes within one minute) in fatal cases. Opisthotonus was regularly observed with intravenous injection, in addition to the signs of central nervous stimulation seen with subcutaneous and intramuscular injection. That the ether anesthesia preceding intravenous injection affected the results observed is not unlikely.

The effects of intocostrin in guinea pigs and mice were substantially the same as in rats, except that (1) larger doses were needed to produce the effects (Table I) and (2) the time to the appearance of the signs of poisoning was longer. Subcutaneous injection into mice resulted in poor absorption and few symptoms, and somewhat larger intramuscular doses were required in mice than in guinea pigs. Intramuscular injection of intocostrin into two cats resulted in greater muscular relaxation and less marked stimulation than in rats, mice, and guinea pigs. The cats survived until six to eight units per

TABLE I. A. SUSCEPTIBILITY OF DIFFERENT SPECIES TO INTOCOSTRIN; B. SUSCEPTIBILITY OF MICE TO CRYSTALLINE D-TUBOCURARINE CHLORIDE CONTAINING 1 STANDARD INTOCOSTRIN UNIT PER 0.162 MG.

	DOSAGE UNITS PER KILOGRAM									
	0.5	0.75	1.0	1.25	1.50	2.0	3.0	4.0	5.0	6.0 OR MORE
A. Intocostrin										
Rats										
Subcutaneous or intramuscular	*C* 0/2 *F* 0/2	*C* 0/2 *F* 0/2	*C* 0/6 *F* 0/6	*C* 2/2 *F* 0/2	*C* 5/5 *F* 1/5	*C* 40/40 *F* 38/40				
Intraperitoneal					*C* 2/2 *F* 0/2	*C* 5/5 *F* 5/5				
Intravenous	*C* 1/5 *F* 1/5		*C* 2/8 *F* 1/8	*C* 4/4 *F* 4/4	*C* 4/4 *F* 4/4	*C* 5/5 *F* 5/5				
Guinea pigs										
Subcutaneous or intramuscular						*C* 2/2 *F* 0/2	*C* 3/3 *F* 3/3			
Cats										
Intramuscular						*C* 0/2 *F* 0/2		*C* 0/1 *F* 0/1		*C* 2/2 *F* 2/2
Rabbits										
Intramuscular						*C* 0/2 *F* 0/2		*C* 0/1 *F* 0/1		*C* 2/2 *F* 2/2
Intravenous			*C* 0/2 *F* 0/2	*C* 7/7 *F* 4/7	*C* 3/3 *F* 2/3	*C* 3/3 *F* 3/3				
Mice										
Intramuscular			*C* 0/2 *F* 0/2			*C* 0/2 *F* 0/2	*C* 3/4 *F* 2/4	*C* 3/3 *F* 1/3	*C* 3/3 *F* 3/3	
B. d-Tubocurarine										
Mice										
Intramuscular							*C* 2/5 *F* 1/5		*C* 3/3 *F* 3/3	*C* 3/3 *F* 3/3

Under each dosage is entered the ratio (*C*) of the number of animals exhibiting convulsions to the number of animals given that dosage and the ratio (*F*) of the number of animals killed to the number given that dosage.

kilogram were administered. Rabbits were somewhat more resistant than cats to intramuscular intocostrin, from eight to ten units per kilogram being the fatal dose. Rabbits manifested few, if any, signs of stimulation with this route of administration until marked asphyxia developed.

Intravenous administration, however, produced in rabbits qualitatively the same effects as subcutaneous or intramuscular administration in rats, provided a sufficient dose was given. With 1 unit per kilogram intravenously, marked

muscular relaxation, as seen in the head drop method for the standardization of intocostrin, was accompanied by no signs of stimulation, and such relaxation was invariably the earliest effect of larger (stimulant) doses. With 1.25 units per kilogram the relaxation was noted in eight to thirty seconds in the seven rabbits given this dose and was followed in another ten to one hundred seconds by cyanosis with respiratory disturbance, muscular twitchings, increased excitability, and clonic convulsions, especially severe when provoked by stimulation. Convulsive movements were noted in some of these animals before cyanosis, as in rats; more often a slight degree of cyanosis was evident before or at the same time as signs of stimulation. The cyanosis became prominent with the onset of violent convulsions. As shown in Table I, the fatal intravenous dose for rabbits was about the same as the fatal subcutaneous or intramuscular dose for rats, but the rabbits died somewhat more quickly (usually within ten minutes).

Crystalline d-tubocurarine was injected intramuscularly into eleven mice, and the results are embodied in Table I(B). Expressed in units, the convulsant and fatal activities of this form of the alkaloid do not differ significantly from those of intocostrin in mice. The crystalline form produced qualitatively the same effects, including the stimulant ones, at about the same times as intocostrin inself in mice and in a few rats and guinea pigs into which it was injected subcutaneously or intramuscularly.

Antagonists to Intocostrin.—Various efforts to antagonize the effects of intocostrin and the results of these efforts are presented in Tables II and III.

Artifical respiration instituted after the failure of normal respiration prolonged the lives of fatally poisoned rats and rabbits and, if continued long enough to permit detoxification of the drug by the body, would presumably have saved them (Table II). Spontaneous respiration was restored at intervals in these animals, and convulsive movements or frank convulsions occurred during these intervals. All six of the animals given artificial respiration died, as did ten rats to which oxygen or 95 per cent O_2–5 per cent CO_2 was administered both before and after the intramuscular injection of intocostrin (2 units per kilogram). Although administration of the gas was begun some time before the respiration was affected, the course of the poisoning was not modified in the slightest by this treatment (Table II).

Into two rats given the fatal dose of intocostrin by intramuscular injection (2 units per kilogram), neostigmine was injected a few minutes after the onset of violent convulsions, as soon as the respiration became abnormal (Table II). Normal respiration was restored promptly, and the animals were able to get up within ninety seconds and to struggle forward. Trembling, mild convulsions with movement, and violent convulsions following external stimulation continued for an hour after the neostigmine was injected, decreasing in intensity with time. The animals survived.

The indications that central nervous stimulation participated in the toxic effects of intocostrin suggested the use of a central nervous depressant to antagonize these effects. Sodium amytal was tried and found to save the lives of 60 per cent of the rats subsequently given the subcutaneous or intramuscular

TABLE II. RESULTS OF VARIOUS FORMS OF TREATMENT OF INTOCOSTRIN POISONING

TREATMENT	ANIMALS	DOSAGE INTOCOSTRIN (UNITS PER KILOGRAM)	RESULTS	REMARKS
Artificial respiration after failure of normal respiration	Rats	2	*C* 4/4 *F* 4/4	Spontaneous respiration restored for brief intervals by treatment; convulsions occurred during these intervals; death followed omission of treatment from twenty to thirty minutes after artificial respiration was first administered
	Rabbits	125	*C* 2/2 *F* 2/2	
100% O_2 or 95% O_2–5% CO_2				
Before and after intocostrin administered	Rats	2	*C* 10/10 *F* 10/10	Course of poisoning exactly as in untreated rats
Cyclopropane (0–30% in O_2)				
Before and after intocostrin administered	Rats	1.5	*C* 0/1 *F* 1/1	Concentration of cyclopropane 30% initially, decreased after anesthesia produced; convulsions appeared whenever anesthesia was sufficiently light; level of anesthesia varied as required to prevent convulsions on the one hand or marked depression of respiration on the other; spontaneous respiration was maintained until death in fifteen to eighty minutes
	Rats	2	*C* 0/3 *F* 3/3	
Immediately after intocostrin administered	Rats	2	*C* 0/3 *F* 3/3	
From 5 to 20 minutes after intocostrin following onset of violent convulsions	Rats	2	*C* 0/2 *F* 2/2	
Neostigmine methyl sulfate ($\frac{1}{4000}$)				
Injected intramuscularly in small portions (total 1 mg. per kilogram) following onset of violent convulsions and impaired respiration	Rats	2	*C* 2/2 *F* 0/2	Convulsions or other signs of stimulation persisted for an hour after neostigmine, decreasing in intensity with time

See Table III for results with sodium amytal. Symbols as in Table I. Intocostrin was administered subcutaneously or intramuscularly to rats, intravenously to rabbits.

TABLE III. PROTECTION OF RATS GIVEN INTOCOSTRIN (2 UNITS PER KILOGRAM SUBCUTANEOUSLY OR INTRAMUSCULARLY) BY INJECTION OF SODIUM AMYTAL (FROM 50 TO 75 MG. PER KILOGRAM INTRAPERITONEALLY)

	TOTAL NUMBER OF RATS	PER CENT SHOWING CONVULSIONS			PER CENT KILLED	AVERAGE TIME TO CESSATION OF RESPIRATION IN FATAL CASES	
		NONE	MILD	SEVERE		MINUTES	STANDARD ERROR
Intocostrin alone	40	0	0	100	95	15.6	±1.4
Intocostrin preceded by sodium amytal	50	67	33	0	40	30.3	±6.1
Intocostrin followed by sodium amytal	10	100 after sodium amytal	–	100 before sodium amytal	100	–	–

dose of intocostrin (2 units per kilogram) fatal to 95 per cent given no depressant (Table III, column 6). Statistical analysis shows the difference between the per cent mortality in the control series and that in the amytal-treated series to be highly significant. All of the survivors lived and were normal for the fortnight of observation following the experiment.

The rats given sodium amytal either before or after intocostrin all manifested complete muscular relaxation early in the poisoning, in contrast to animals given no depressant in which complete muscular relaxation appeared terminally only, if at all. Death, when it occurred in the amytalized rats, followed a prolonged period of flaccid paralysis and nonresponsiveness. In rats given the depressant after convulsions had developed, the convulsions became less violent and finally ceased, but the animals nevertheless died. Possibly they lived somewhat longer than if they had not received sodium amytal. The ability of the depressant to prolong life is more certainly shown in the series of animals injected with sodium amytal before intocostrin; the rats in this series, which were not saved by the sodium amytal, lived on the average twice as long after the intocostrin was administered as the unprotected rats did (Table III, column 7).

Because intocostrin is known to be useful clinically in anesthesia with cyclopropane, an attempt was made to protect rats against a fatal dose of intocostrin by means of this anesthetic (Table II). It was found possible to keep the rats alive for over an hour and free from convulsions during this time, whether cyclopropane was started before or after intocostrin was administered. However, all of the animals eventually died under the anesthetic or following convulsions which developed when the anesthetic was removed. Experience with the method and consequent improvements in technique might show cyclopropane to be an effective antidote to intocostrin.

DISCUSSION

Interference with the passage of impulses from nerves to muscles, typical curare action, is taken to be the pharmacodynamic action of d-tubocurarine (and therefore of intocostrin which contains tubocurarine) that produces weakness, muscular relaxation, and paralysis in mammals. Involvement of the respiratory muscles in this effect certainly accounts in part for the fatal asphyxia, as shown by the lifesaving ability of neostigmine in our rats and in the rabbits given d-tubocurarine in fatal doses intravenously (1.5 standard intocostrin units per kilogram) by Koppanyi and Vivino.[4] These observations with neostigmine are in agreement with much earlier work on anticholinesterases as antidotes to curare. The curarization is obviously far from complete in all the mammals used in our experiments. In spite of the current view that the respiratory muscles are the last to be affected by curare, curarization of the respiratory muscles does not seem to be less complete than that of the other skeletal muscles. The two following reports from the literature on curarine in rats are pertinent in this connection. Blume[5] was unable to achieve neuromuscular paralysis (tested by electrical stimulation of sciatic nerves) even with near-fatal doses.

West[1, p. 86] found that with electrical stimulation of the nerves conduction from the phrenic nerve to respiratory muscles might outlast conduction from the sciatic nerve to leg muscles or might disappear before the latter.

Stimulation of the central nervous system, a second, much less familiar action of curare, is probably also a factor in the action of d-tubocurarine or intocostrin in mammals. Phenomena attributed to central stimulation (muscular spasms, convulsions) have been observed consistently following direct application of curare preparations to the central nervous system (frogs, dogs).[6, 7] That the underlying mechanism is actually stimulation of nervous tissue has been established by Eccles' recent report[8] on the effect of curarine on the spinal cord and roots isolated from the frog. Under the influence of curarine in a concentration twice as great as necessary to block neuromuscular transmission, both the synaptic potential of the cord and the discharge of impulses set up by a single dorsal root volley were found to be prolonged. With much higher concentrations continuous electrical activity occurred spontaneously. The actions of curarine on the electrical behavior of the cord therefore resemble those of strychnine and are the reverse of curarine effects at sympathetic ganglia and at junctions between motor nerves and skeletal muscles. Our preliminary experiments with intocostrin injected into the cerebrospinal canal of frogs demonstrate that intocostrin like other curare preparations stimulates the central nervous system when applied directly to it.

Signs of stimulation have also frequently been observed when curare was administered by methods other than direct application to the central nervous system. Blume produced convulsions and other signs of stimulation in rats, mice, guinea pigs, and, in a milder degree, even in cats by the subcutaneous or intravenous administration of curarine or calabash-curare.[5] West observed stimulation of several mammalian species with various curare preparations and came to the conclusion that central nervous excitation is an action of certain forms of curare, although apparently not of curarine or d-tubocurarine.[1] That tubocurarine nevertheless actually possesses stimulant activity is strongly indicated by the recent work of McIntyre, Dunn, and Tullar.[9] They report increased electrical activity of the brains of dogs given this alkaloid intravenously. The increase, although "transient," appears to be considerable and may affect either voltage or frequency. The succeeding period of depressed activity in these animals recalls the observations of Pick and Unna[10] on the brains of pithed frogs given d-tubocurarine into the lymph sac. They observed inbitition and suppression of brain electrical activity with this and other curarizing substances; however, inasmuch as strychnine and picrotoxin failed to have any stimulant effect in similar frog experiments, these observations do not contradict the view that intocostrin stimulates the central nervous system of mammals directly.

It may be argued that the signs of central stimulation observed following the administration of curare preparations by other routes than direct application to the central nervous system are in fact due not to drug action on this system but to the production of asphyxia by the curarization of respiratory

neuromuscular junctions. This interpretation, although found in the literature,[11, 12] is difficult to defend in view of (1) the stimulant activity of directly applied curare, (2) the limited degree to which classical curarization occurs in mammals, and (3) the presence of stimulation in some cases in the absence of overt asphyxia.

That direct central nervous stimulation plays a significant role in the action of intocostrin administered in large enough dosage by any route to mammals is indicated by (1) the ability of sodium amytal to prolong or save the lives of rats given a fatal dose of this form of curare, (2) persistence of signs of central nervous stimulation in rats after antagonism of the peripheral neuromuscular effects of neostigmine, and (3) lower fatal dosage for species responding to the drug with marked stimulation (rats, guinea pigs) than for species responding with marked relaxation (cats, rabbits). Some of the species difference is associated with difference in rate of absorption of the drug from the tissues, but the difference in the size of the fatal intravenous dose for rats, and rabbits, for instance, is probably associated with the greater ease of producing convulsions in the former.

Since the protective effect of neostigmine affords evidence that neuromuscular depression also plays a significant role in the toxic action of intocostrin, it is assumed that both central stimulation and neuromuscular depression contribute to the intocostrin asphyxia fatal to mammals. The asphyxiating power of depression of respiratory neuromuscular junctions is obvious. Central stimulation by producing convulsions would greatly increase the oxygen used by the body (perhaps three- or fourfold if the convulsions were equivalent to severe exercise) at a time when the respiratory apparatus was so incapacitated by the peripheral effect of the intocostrin as to be hardly able to meet the basal oxygen requirements. Moreover, convulsive waves of activity in the brain doubtless interfere with the orderly transmission of respiratory impulses and therefore lead to inadequate respiratory movements or respiratory arrest. Any agent that prevented convulsions would therefore decrease the degree of asphyxia, and anyone of many central depressants might be expected to protect animals against a fatal dose of intocostrin.

Koppanyi and Vivino[4] observed that ephedrine and tyramine, although themselves useless as antidotes to d-tubocurarine, when combined with physostigmine or neostigmine, protected rabbits against 1.33 to 2 fatal doses of the curare alkaloid. Physostigmine or neostigmine alone had no protective value against more than one fatal dose (1.5 units per kilogram).[4] The mechanism by which the long-lasting sympathomimetic agents produce their effect is unknown.

The classification of curare convulsions as strychninelike by various workers seems to us incorrect for mammals; McGuigan's[7] statement that the convulsions resemble those produced by picrotoxin more than those produced by strychnine agrees with our experience. In McGuigan's experiments as in ours and in all others described in any detail in the curare literature, the character of the convulsions indicates that, in contrast to strychnine, the curare alkaloids

produce convulsions in mammals by stimulation at a central nervous system level higher than the spinal cord.

Our experimental results reinforce the frequent but not superflous warning against the use of intocostrin in patients without having immediately available effective means (artificial respiration, neostigmine) for combating respiratory disturbances. In addition, our results suggest that some means of producing central depression should also always be available when intocostrin is to be used clinically. Although it is clear from the size of the dose recommended for clinical use (from 1.1 to 3.3 units per kilogram, intravenously or intramuscularly) that man must be relatively insensitive to the stimulant effects of d-tubocurarine (see also West,[1, p. 83]) signs of stimulation (muscle twitchings, reflex activation) have been reported with intocostrin in unanesthetized patients[13] and incompletely anesthetized patients.[14] That these signs are not entirely asphyxial is shown by clinical success in controlling them with increased anesthesia. Certain it is that such signs do not indicate the need for more intocostrin. Griffith believes that in large doses intocostrin has some central action in man, but he does not specify the nature of the action.[15]

In addition to overcoming any central stimulation produced by intocostrin, the use of a central depressant has the advantage of increasing the degree of muscular relaxation resulting from a given intocostrin dose. In animals, muscular relaxation with intocostrin is incomplete unless a central nervous depressant is used; evidently central depression makes an important though obscure contribution to "curarization" of skeletal muscle by intocostrin. In unanesthetized patients, likewise, complete muscular relaxation is not ordinarily observed as the result of the peripheral action of intocostrin, although it has been reported with large intravenous doses causing unconsciousness.[13] Cullen, who was unable to obtain sufficient abdominal muscle relaxation to permit satisfactory examination of a patient without premedication even with the intravenous injection of an amount of intocostrin that resulted in respiratory paralysis,[12] regards properly conducted inhalation anesthesia as essential for the successful and safe production of complete muscular relaxation with intocostrin.[16] Mallinson[18] considers anesthesia as deep as the second plane of the surgical stage requisite for maximum abdominal relaxation when intocostrin is used.

For two reasons, therefore, to antagonize any central stimulation and to contribute towards muscular relaxation, means for depressing the central nervous system would appear to be potentially valuable whenever intocostrin is to be used. Our experiments do not indicate which would be the most useful depressants for this purpose. They do however suggest that sodium amytal should be used with great caution, if at all, in view of the toxicity to rats of the combination of sodium amytal with intocostrin. The cause of the toxicity was not investigated; it may be related to one or several of the known side actions of amytal in the body. For surgical operations, intocostrin is frequently combined with one of the inhalation anesthetics or with sodium pentothal. Possibly one of these should be the depressant available for use if needed with intocostrin in other cases. However, two of these depressants have been shown to possess

curariform activity (ether in considerable degree, sodium pentothal slightly)[17] and so are not unobjectionable. The known central stimulant action of morphine makes it unsuitable likewise.

SUMMARY

1. Intocostrin or crystalline d-tubocurarine chloride, injected in sufficient dosage subcutaneously, intramuscularly, intraperitoneally, or intravenously into rats, mice, guinea pigs, rabbits, and cats, produces hyperexcitability and clonic convulsions in addition to partial curarization.

2. Signs of central nervous stimulation are more conspicuous in some species than others and are shown by rats without signs of asphyxia. Species (rats, guinea pigs, mice) manifesting much stimulation are killed by smaller doses of the drug than species (rabbits, cats) manifesting more curarization.

3. With fatal doses, death in all these animals is due to asphyxia, attributed in part to curarization of respiratory neuromuscular junctions peripherally and in part to stimulation centrally. The asphyxia may be relieved by neostigmine (peripheral action) or by sodium amytal (central action). Sodium amytal protects 60 per cent of rats from the effects of a dose of intocostrin (2 units per kilogram) fatal to 95 per cent of untreated rats. The other 40 per cent of rats given sodium amytal prior to intocostrin live twice as long after the intocostrin injection as those not given sodium amytal.

4. Central nervous depressants (sodium amytal, cyclopropane), decrease, abolish, or prevent intocostrin convulsions. Reduction of asphyxia by oxygen administration or artificial respiration is less, if at all, effective in controlling convulsions.

5. The conclusion from these observations that central nervous stimulation is one of the pharmacodynamic actions of intocostrin is in agreement with the conclusions of other workers using other curare preparations.

6. The flaccidity and decrease of spontaneous movements manifested by mammals given intocostrin or d-tubocurarine alone do not develop into complete neuromuscular paralysis until just before death. In animals given a central nervous depressant in addition to intocostrin muscular relaxation becomes complete at an early stage of intocostrin action.

7. Certain central nervous system depressants may be useful adjuncts to intocostrin to combat central stimulation as well as to increase muscular relaxation.

The author would like to express her sincere appreciation to Dr. Helen Graham, for her aid and advice in this research.

REFERENCES

1. West, Ranyard: A Pharmacological Study of Derivatives of Two Specimens of Tubo-Curare, and an Examination of Four Members of Genus Strychnos and One Rubiaceous Plant Associated With the Curares of British Guiana, Arch. internat. de pharmacodyn. et de thérap. **56**: 81-116, 1937.
2. King, Harold: Curare Alkaloids. I. Tubocurarine, Journal of the Chemical Society 1381-1389, 1935.
3. Bennett, A. E.: Curare: a Preventive of Traumatic Complications in Convulsive Shock Therapy, Am. J. Psychiat. **97**: 1040-1060, 1941.

4. Koppanyi, T., and Vivino, A. Earl: Prevention and Treatment of d-Tubocurarine Poisoning, Science **100:** 474-475, 1944.
5. Blume, Wilhelm: Die Krampfwirkung des Curarins, Arch. f. exper. Path. u. Pharmakol. **175:** 745-753, 1934.
6. Tillie, Joseph: Ueber die Wirkungen des Curare und seiner Alkaloide. Parallelle zwischen den Wirkungen des Curarins und Methylstrychnins, Arch. f. exper. Path. u Pharmak. **27:** 1-38, 1890. References to similar earlier work will be found here.
7. McGuigan, Hugh: The Central Action of Curare, J. Pharmacol. & Exper. Therap. **8:** 471-477, 1916.
8. Eccles, J. C.: Synaptic Potentials of Motoneurones, J. Neurophysiol. **9:** 87-120, 1946.
9. McIntyre, A. R., Dunn, A. L., and Tullar, P. E.: The Effect of d-Tubocurarine on the Electrical Activity of Dogs' Brains, Federation Proceedings (American Societies for Experimental Biology) **5:** 67, 1946.
10. Pick, E. P., and Unna, K.: The Effect of Curare and Curare-like Substances on the Central Nervous System, J. Pharmacol. & Exper. Therap. **83:** 59-70, 1945.
11. Perlstein, M. A., and Weinglass, A.: Fatal Effects of Prolonged Complete Curarization, Am. J. Dis. Child. **67:** 360-364, 1944.
12. Cullen, Stuart C.: The Use of Curare for the Improvement of Abdominal Muscle Relaxation During Inhalation Anesthesia, Surgery **14:** 261-266, 1943.
13. Whitacre, R. J., and Fisher, A. J.: Clinical Observations on the Use of Curare in Anesthesia, Anesthesiology **6:** 124-130, 1945.
14. Harroun, P., Bechert, F. E., and Hathaway, H. R.: Curare and Nitrous Oxide Anesthesia for Lengthy Operations, Anesthesiology **7:** 24-28, 1946.
15. Griffith, Harold R.: Physiological and Clinical Action of Curare, Current Researches in Anesth. & Analg. **25:** 45-51, 1946.
16. Cullen, Stuart C.: Clinical and Laboratory Observations on the Use of Curare During Inhalation Anesthesia, Anesthesiology **5:** 166-173, 1944.
17. Gross, E. G., and Cullen, Stuart C.: The Effects of Anesthetic Agents on Muscular Contration, J. Pharmacol. & Exper. Therap. **78:** 358-365, 1943.
18. Mallinson, F. Barnett: Curare in Anesthesia, Lancet **2:** 75-76, 1945.

HEMOGLOBIN LEVELS IN SPECIFIC RACE, AGE, AND SEX GROUPS OF A NORMAL NORTH CAROLINA POPULATION*

D. F. Milam and Hugo Muench
New York, N. Y.

A STUDY of the distribution of hemoglobin levels in a population may furnish important information regarding its nutritional status. At the same time, the expected frequency of occurrence of different hemoglobin values in a presumably normal population should be delimited in order to indicate those which may have a pathologic basis.

During the years 1940-1945 large population groups in North Carolina were studied to determine their nutritional status. In the course of these surveys a careful estimate of hemoglobin level was made for each of 3,029 persons, 2,168 of whom were white and 861 (28.4 per cent) colored. This racial distribution represents fairly closely that of the area studied and that of the state as a whole. The surveys were conducted in Chatham, Alamance, Orange, and Wayne counties. With the exception of Wayne, all of these counties lie in or near the fall line just west of the coastal plain. The results of the studies have appeared in several publications.[1-3] The samples selected for the study comprise families chosen to represent as nearly as possible the population of the county in question.

Wayne County, in the midcoastal plain, was the only one in which hookworm infection or malaria might affect the "normality" of the hemoglobin values. Extensive hookworm studies recently made there[4] showed a light infestation of some 20 per cent of the population. Of the blood films taken throughout the year of study in this county and examined by the malaria section of the North Carolina State Board of Health, only one showed malaria parasites. No other disease was recognized which might have unduly influenced the hemoglobin levels. It may be assumed, therefore, that the population studied provides a fair picture of hemoglobin values for a normal population from this area.

METHOD

Evelyn's method[5] of determining hemoglobin by the photoelectric colorimeter was employed. Blood samples of approximately 30 c.c. were oxalated when drawn in small bottles with rubber stoppers, and hemoglobin tests were made some two to four hours later. At this time the blood components were remixed by inverting the bottle a minimum of fifteen times, and the pipette was filled from the middle of the bottle. Red cell counts and hematocrit and hemoglobin determinations were then made. One-tenth cubic centimeter of whole blood was added to a flask containing 50 c.c. of distilled water, the pipette being thoroughly rinsed therein. Ten cubic centimeter aliquots, each with one drop of

Received for publication, June 5, 1946.

*The observations on which this paper is based were a joint activity of the North Carolina State Board of Health, Duke University School of Medicine, and the International Health Division of The Rockefeller Foundation.

concentrated ammonia added and mixed, were examined within ten minutes in the Evelyn colorimeter with 540 filter and 10 mm. aperture and dim light. The galvanometer readings were converted into L values from Evelyn's tables. The L values were multiplied by 100/2.58 to obtain estimates of grams of hemoglobin per 100 c.c.

STATISTICAL TREATMENT

The examined population was subdivided by color and sex into two age groups, differentiating persons under 12 from those who were 12 and over. There are, therefore, eight subgroups exhibiting certain differences for consideration. In Figs. 1 and 2 are shown graphically the distribution of hemoglobin values for each subgroup. To facilitate comparison the frequencies pertaining to each distribution have been converted to percentages of the total number of persons in the subgroup. These are plotted on the vertical scale of the histogram. On the base scale the amounts of hemoglobin in grams per 100 c.c. of blood are entered for the mean of the group and for various multiples of the standard deviation.

In Table I are listed the values of the means and standard deviations for each subgroup, together with their standard errors. Also included in Table I are the limiting hemoglobin values for each distribution, between which half of

TABLE I. DISTRIBUTION CONSTANTS OF HEMOGLOBIN LEVELS FOR WHITE AND COLORED POPULATIONS BY AGE AND SEX, AND WITH LIMITS BETWEEN WHICH ONE-HALF AND NINETEEN-TWENTIETHS OF THE OBSERVATIONS MAY BE EXPECTED TO FALL

		WHITE		COLORED	
		NUMBER	GRAMS OF HEMOGLOBIN PER 100 C.C.	NUMBER	GRAMS OF HEMOGLOBIN PER 100 C.C.
Mean					
Under 12	Boys	357	12.67 ± 0.046	193	11.89 ± 0.078
	Girls	362	12.54 ± 0.046	188	12.13 ± 0.062
12 and over	Men	611	14.25 ± 0.049	212	13.76 ± 0.086
	Women	838	12.87 ± 0.043	268	12.16 ± 0.087
	Women (fitted curve)	796	13.08	236	12.64
Standard deviation					
Under 12	Boys		0.867 ± 0.032		1.082 ± 0.055
	Girls		0.874 ± 0.032		0.845 ± 0.044
12 and over	Men		1.209 ± 0.035		1.249 ± 0.061
	Women		1.254 ± 0.031		1.430 ± 0.062
	Women (fitted curve)		0.911		0.815
Limits of one-half distribution					
Under 12	Boys		12.09 - 13.25		11.16 - 12.62
	Girls		12.01 - 13.11*		11.56 - 12.70
12 and over	Men		13.43 - 15.07		12.92 - 14.60
	Women		12.02 - 13.72		11.20 - 13.12
	Women (fitted curve)		12.48 - 13.70		12.09 - 13.19
Limits of nineteen-twentieths of distribution					
Under 12	Boys		10.97 - 14.37		9.77 - 14.01
	Girls		10.95 - 14.17*		10.47 - 13.79
12 and over	Men		11.88 - 16.62		11.31 - 16.21
	Women		10.41 - 15.33		9.36 - 14.96
	Women (fitted curve)		11.30 - 14.88		11.04 - 14.24

*First individual (hemoglobin, 6.5 Gm.) omitted.

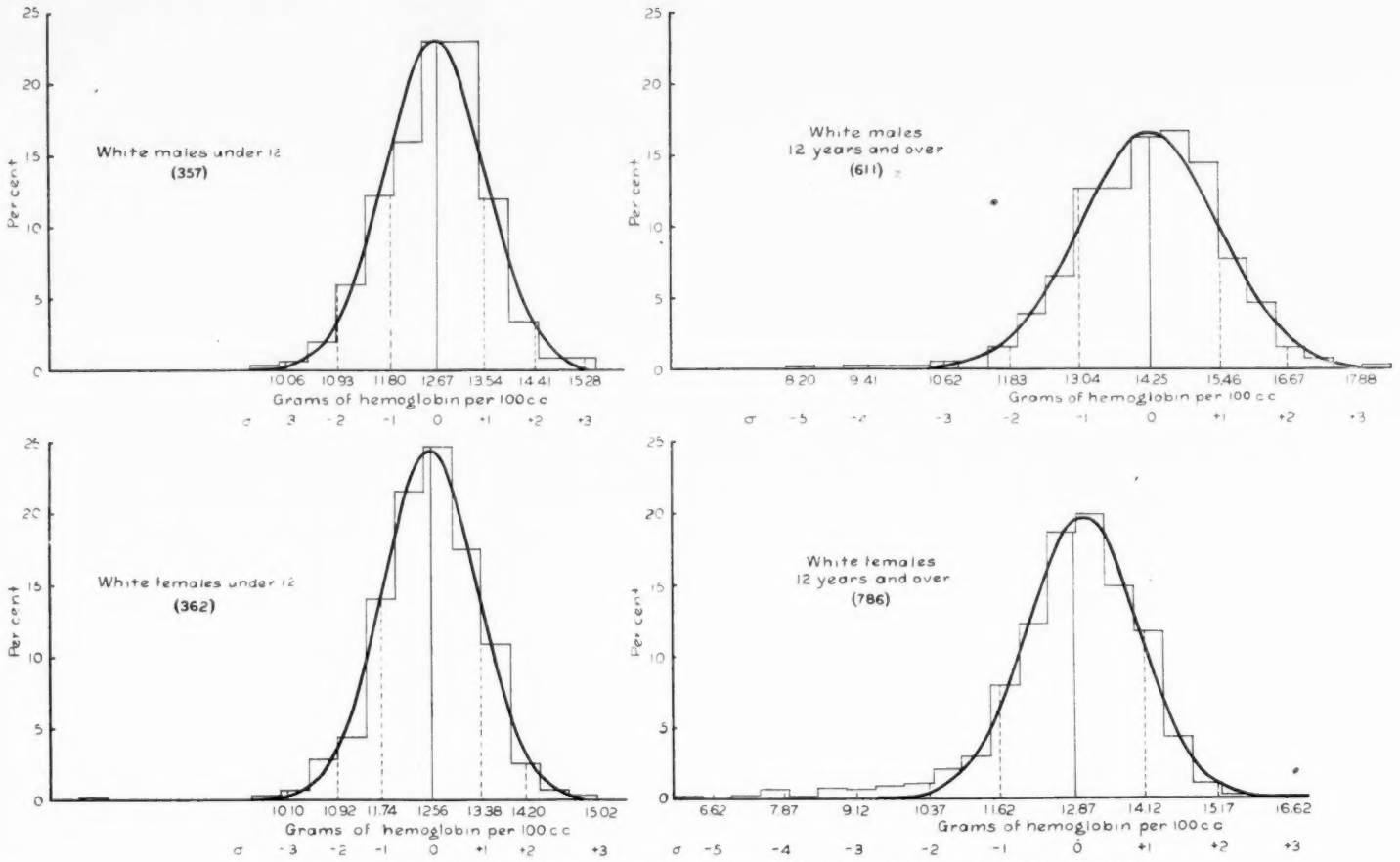
White males under 12
(357)
Per cent
Grams of hemoglobin per 100 c c
10.06 10.93 11.80 12.67 13.54 14.41 15.28
σ -3 -2 -1 0 +1 +2 +3
White males
12 years and over
(611)
Per cent
Grams of hemoglobin per 100 c c
8.20 9.41 10.62 11.83 13.04 14.25 15.46 16.67 17.88
σ -5 -4 -3 -2 -1 0 +1 +2 +3
White females under 12
(362)
Per cent
Grams of hemoglobin per 100 c c
10.10 10.92 11.74 12.56 13.38 14.20 15.02
σ -3 -2 -1 0 +1 +2 +3
White females
12 years and over
(786)
Per cent
Grams of hemoglobin per 100 c c
6.62 7.87 9.12 10.37 11.62 12.87 14.12 15.17 16.62
σ -5 -4 -3 -2 -1 0 +1 +2 +3

Fig. 1.—Percentage frequency distributions of hemoglobin determinations of white persons.

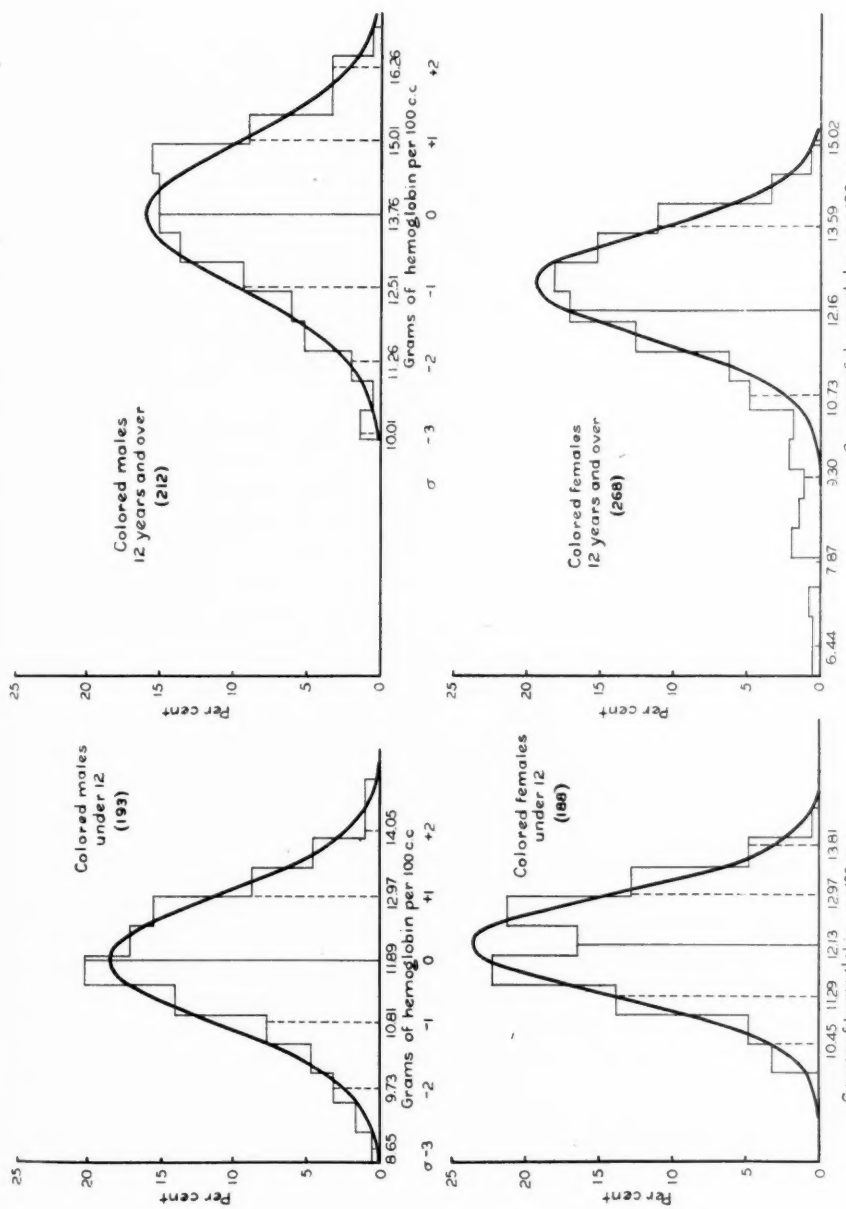

Fig. 2.—Percentage frequency distributions of hemoglobin determinations of colored persons.

the observed values may be expected to fall, as well as the limits between which nineteen-twentieths of the expected values will lie. Individual observations falling outside the latter limits would be rare and therefore under suspicion of being abnormal.

The histograms for six of the distributions shown in Figs. 1 and 2 are fitted with normal frequency curves having the means and standard deviations of the observed distributions. It is apparent that these curves are good expressions of the observed occurrence of hemoglobin values and may be used to express the probability of obtaining other observations in such groups between any given values. It is obvious, however, that when such curves are applied to the data for the two groups of women, although they describe the distribution of the great majority of the observations, they fail to take into account a number with very low values. Among white adult females 42 of the 838 individuals (5 per cent) fall below and outside the normal curve fitted to the bulk of the observations, and among colored women 32 of 268 (12 per cent) fall below the lower limit set by the fitted curve.

None of the usual frequency distributions of the Pearsonian series gave an adequate picture of the observations pertaining to the two groups of women. Finally, normal frequency curves were fitted to approximately three-quarters of the observations at the upper end of the distribution by the method of incomplete moments[6] and the curves then extended through the lower ends of their ranges. As indicated in Figs. 1 and 2, there is an excess of observations for each of the two groups over the expected number at the lower end of the hemoglobin scale.

In Table I two sets of means and standard deviations are given for each group of women. Those derived from all the observations are entered first and those from the fitted curve, second. The means and standard deviations plotted in Figs. 1 and 2 are the ones pertaining to all observations.

DISCUSSION OF RESULTS

It appears that, in general, the hemoglobin values encountered in the subgroups of this surveyed population can be expressed in terms of a so-called normal frequency distribution. This is strictly true for men and for children of both sexes with the exception of one girl with 6.5 Gm. of hemoglobin per 100 c.c., which was completely outside the range of expected values. From 90 to 95 per cent of women have values which are well described by the normal curve, with the remaining observations falling below the expected range. The fact that this divergence is confined, with one exception, to women suggests that the apparent deficiency is linked with the reproductive processes, possibly with the menstrual cycle. At any rate, low hemoglobin values may be encountered occasionally in women without arousing the suspicion of an acute disease process. In a survey of hemoglobin levels in Great Britain in 1943[7] the values of 1 per cent of men were found to fall below 80 (Haldane-Gowers hemoglobinometer reading), while those of 6 per cent of single women and 10 per cent of married women fell below this level, which tends to confirm the observations reported in this paper.

In Table I it is indicated that the average hemoglobin values for white persons in all groups are higher than the corresponding ones for colored persons, and the difference in each instance is statistically significant. Sex differences in average level are negligible for children, but men have considerably more hemoglobin than do women, even if the very low values pertaining to the latter are omitted. Men have a higher average level than do boys, but the average for women is only slightly higher than that for girls.

The dispersion of the observations, as measured by the standard deviation, is greater among adults, both males and females, than it is among children. The scatter of the values under the fitted curves for women, however, is similar to that for girls. If the fitted curve may be considered, an expression of the distribution of hemoglobin values not affected by the factor or factors tending to pull down the level, there is little difference between the distributions of girls and women or between those of boys and women. Men, however, present definitely higher levels which are accompanied by a wider scatter of the individual observations.

Again it may be concluded from Table I that only rarely will a normal white child be encountered in such a population with less than 11 Gm. of hemoglobin per 100 c.c. of blood, a white man with less than 12 Gm. or a white woman with less than 10.4 Gm. Corresponding values for a colored population would be from 0.5 to 1 Gm. less.

TABLE II. MEAN DAILY INTAKE OF IRON IN THE DIET (FOUR NORTH CAROLINA COUNTIES, 1940-1945)

LOCATION AND DATE	NUMBER OF INDIVIDUALS		MILLIGRAMS OF IRON	
	WHITE	COLORED	WHITE	COLORED
Chatham County, 1940-1941	244	73	8.6	6.3
Wayne County, 1941-1943	933	453	9.4	8.8
Alamance County, 1943-1944	612	139	11.7	11.2
Orange County, 1944-1945	232	0	16.2	
Total	2021	665		

Attempts to raise hemoglobin levels in individuals have not always resulted in a permanent rise, even while curative treatment is being continued.[8] Furthermore, individual variations within a normal range are to be expected, as has been shown here. Consequently, a person with a low reading, particularly if a woman, must be carefully examined before such a value can be assumed to have a pathologic basis. While menstrual blood loss offers a ready explanation for low hemoglobin levels among women, there is need for more definite information regarding the correlation between the amounts of blood lost and hemoglobin readings before this reason can be accepted as valid.

Dietary factors no doubt influence hemoglobin levels. Variation in copper intake as well as that of protein must affect them. There was no apparent protein deficiency in the group here studied since there were only four individuals with less than 6.0 Gm. of plasma protein per 100 c.c.[9]

Dietary intake of iron may be assumed to be the most important factor affecting hemoglobin levels. During the five and one-half years of the survey,

data on iron intake in the four counties were assembled and are summarized in Table II. In every instance the iron intake fell below the recommended allowance, being approximately one-half the amount in one group. Whether this influenced hemoglobin levels is difficult to determine from the data on hand. A discussion of iron intake with respect to hemoglobin level may be found in previous reports.[1-3]

The chief sources of dietary iron in the most recently surveyed group (Orange County) were calculated, and the results for persons 15 years of age and over were as follows[10]:

FOOD	PER CENT OF TOTAL IRON	
	MEN	WOMEN
Dried beans	24.4	24.7
Enriched bread	11.5	8.4
Eggs	10.1	8.8
Lean pork	6.5	6.8
Milk	4.9	3.4
Dried peas	4.1	3.7
Whole cereal	3.8	6.4
	65.3	62.2

It might be assumed that the fondness of southern people for greens such as turnip tops, mustard greens, and collards, would make these the chief sources of iron. Actually the bulk of iron consumed is contributed by foods which are poorer individual sources but are eaten in larger quantities. Beans were the outstanding source for all groups, contributing about a fourth of the total iron intake. For children, whole cereals were the second source with eggs or milk third.

SUMMARY

This paper presents distributions of hemoglobin levels as determined by the Evelyn photoelectric colorimeter in 2,168 white persons and 861 colored persons in four North Carolina counties. Members of the families examined were living at home and apparently in normal health. The examined population was divided into eight subgroups according to race, sex, and age. The mean grams per cent of hemoglobin ranged between 12 and 14. Means for colored persons were from ½ to 1 Gm. below those for corresponding groups of white persons. Means for women of both races were about 1.5 Gm. below those for men. Differences between means for boys and girls were not great for either race. In general, observed values were well described by normal frequency curves, but among women of each race there was a group comprising from 5 to 10 per cent of the total with hemoglobin values lying definitely below and outside the range of the curve fitted to the main group of observations. Calculated dietary iron intakes for the population under observation was somewhat below that usually recommended as a minimum requirement.

REFERENCES

1. Milam, D. F.: A Nutrition Survey of a Small North Carolina Community, Am. J. Pub. Health 32: 406-412, 1942.
2. Milam, D. F., and Anderson, R. K.: Nutrition Survey of an Entire Rural County in North Carolina, South. M. J. 37: 597-605, 1944.

3. Milam, D. F., and Darby, W. J.: The Average Diet of a Southern County and Its Effects on Nutritional Status, South. M. J. **38**: 117-125, 1945.
4. Keller, A. E., Leathers, W. S., and Knox, J. C.: Present Status of Hookworm Infestation in North Carolina, Am. J. Hyg. **26**: 437-454, 1937.
5. Evelyn, K. A.: A Stabilized Photoelectric Colorimeter With Light Filters, J. Biol. Chem. **115**: 63-75, 1936.
6. Pearson, Karl: Tables for Statisticians and Biometricians, Part I, ed. 2, Biometrics Laboratory, London, 1924, Univ. College, pp. XXIV, 22-23.
7. The Committee on Haemoglobin Surveys: Haemoglobin Levels in Great Britain in 1943, M. Research Council, Great Britain, Special Report Series 252, 1945, His Majesty's Stationery Office, pp. 24-25.
8. Fowler, W. B., and Barer, A. P.: Some Effects of Iron on Hemoglobin Formation, Am. J. M. Sc. **201**: 642-651, 1941.
9. Milam, D. F.: Plasma Protein Levels in Normal Individuals, J. Lab. & Clin. Med. **31**: 285-290, 1946.
10. Milam, D. F., and Bell, Mary Parks: Sources of Principal Nutrients in the Diet of a North Carolina Village Population, J. Am. Dietet. A. **22**: 411-414, 1946.

HEMATOLOGIC SURVEY OF REPATRIATED AMERICAN MILITARY PERSONNEL

LIEUTENANT GEORGE E. CARTWRIGHT, MEDICAL CORPS, ARMY OF THE UNITED STATES, AND MAXWELL M. WINTROBE, M.D.,* SALT LAKE CITY, UTAH

INTRODUCTION

THIS report describes a hematologic survey undertaken to determine the incidence, severity, and type of anemia, as well as the status of the leucocytes and platelets, in repatriated American military personnel from the Far East. It was a part of a general survey carried out at Crissy Annex, Letterman General Hospital, San Francisco, by the Board of Medical Survey of Repatriated American Military Personnel.

A hemoglobin determination (Haden-Hausser) was made on each individual during a general medical examination. On the basis of these determinations, the following day a small group of anemic individuals was selected for a more detailed hematologic study. It is this group of seventy-five individuals which is the subject of a more detailed report in this paper.

The hematologic methods used in the study of the seventy-five selected subjects were those described by Wintrobe.[1] Blood was collected in oxalate bottles containing a 2:3 mixture of potassium and ammonium oxalate. Duplicate red counts were done on each sample of blood. If the two counts did not check within twenty-five cells they were repeated. Hemoglobin determinations were made with a Sahli hemoglobinometer. The hematocrit tubes were spun at 3,000 r.p.m. for thirty minutes in a large International type centrifuge. Such a procedure was found to insure maximal packing under the conditions of this study. Using these methods normal indices were obtained on blood from normal individuals. In experiments using heparin as an anticoagulant, no significant difference in these values was observed. Most of the determinations in these seventy-five subjects were done by one of us (G. E. C.) personally. A few were done by a trained experienced technician under supervision. Serum iron determinations were made using the methods of Kitzes, Elvehjem, and Schuette.[2] Using this method we have found the range for serum iron in healthy young men to be from 90 to 150 micrograms per 100 c.c.[3] This is a somewhat narrower range than that reported by several investigators[4] and is probably explained by the fact that our group was restricted to young men. The method of Cartwright, Jones, and Wintrobe[5] was employed for the determination of serum copper. We have found the range for serum copper in healthy young men to be from 90 to 140 micrograms per 100 c.c.[3]

Received for publication, June 7, 1946.

*Department of Medicine, University of Utah School of Medicine.

CLINICAL HISTORIES

The subjects of this study had been prisoners of the Japanese for periods of from thirty-nine to forty-six months with one exception. This individual had been imprisoned for only nine months. The average duration of imprisonment for the group was 40.1 months. With two exceptions all had been imprisoned in both the Philippines and in Japan.

All individuals gave a history of weight loss varying between 5 and 90 pounds. The average weight loss from the time of capture to the time of release was 37 pounds. This figure, however, does not give a true picture of the degree of weight loss, since many men were below their normal weight at the time of capture and since, due to the dropping of supplies from the air prior to release, many men had gained weight by the time of their actual release.

A history of malaria was obtained in 76 per cent of the group. The number of attacks varied from one to sixty. The average number of attacks per individual affected was ten. Most of these occurred in the early period of the war while the subjects were in the Philippines. During the imprisonment in Japan, in most cases, the malaria subsided spontaneously. None of our subjects had had active malaria within four months prior to this study.

The incidence of certain prominent signs and symptoms of nutritional deficiency as obtained by history is indicated in Table I. Pellagra as evidenced by cheilosis, glossitis, stomatitis, dermatitis, and diarrhea had been present in some 50 to 70 per cent. Several features of this pellagra need comment. The incidence was much greater in the Philippines. Only rarely, if at all, did this symptom complex develop in Japan while the men were sporadically receiving soybeans. The incidence of typical pellagrous dermatitis over the exposed parts was relatively infrequent. This was the case in spite of the fact that the men were constantly exposed to considerable sunlight.

TABLE I. INCIDENCE OF CERTAIN SIGNS AND SYMPTOMS AS OBTAINED FROM HISTORY IN SEVENTY-FIVE REPATRIATED AMERICAN MILITARY PERSONNEL FROM FAR EAST

SYMPTOMS AND SIGNS	NUMBER OF CASES	PER CENT
Cheilosis	54	72
Glossitis	51	68
Stomatitis	59	65
Scrotal lesions	30	40
Pellagrous dermatitis	19	25
Diarrhea	39	52
Edema	58	77
Burning of the feet	35	46
Hyperesthesia of the feet	37	50
Paresthesia of the feet	38	51
Bleeding gums	11	15
Petechiae	1	1
Ecchymosis	2	3
Night blindness	5	7
Photophobia	9	12
Diminution of vision	13	17
Burning of eyes	12	16
Excessive lacrimation	9	12
Weight loss	75	100
Weakness	75	100
Lassitude	75	100

The occurrence of a scaly, sometimes an erythematous weeping scrotal, dermatitis accompanied by extreme tenderness of the scrotum, and in some cases associated with edema, was relatively common. In some cases scrotal tenderness without dermatitis occurred. About 55 per cent of the individuals with a history of glossitis and stomatitis gave a history of scrotal dermatitis. In only one subject did scrotal dermatitis occur in the absence of glossitis and stomatitis. In three cases glossitis and stomatitis developed in the absence of cheilosis. Most generally when glossitis occurred stomatitis also was present. Severe and persistent diarrhea accompanying glossitis and stomatitis was not as frequent as expected. Many, in fact most of the individuals, had short bouts of diarrhea lasting only a few days. In Table I only persistent diarrhea is recorded. No cases of diarrhea resembling sprue were found.

One individual with a severe glossitis gave a history of having received an injection of niacin. Within three hours the burning of the tongue ceased. Another individual with severe diarrhea and dermatitis stated that these symptoms disappeared within two weeks following niacin therapy.

Beriberi was exceedingly prevalent in the group and occurred in both Japan and in the Philippines. A history of wet beriberi was obtained in 77 per cent and a history of dry beriberi in about 50 per cent. Many individuals had had both the wet and the dry types of beriberi, but the most striking feature was that in no individual questioned was a history of both types occurring simultaneously obtained. Often when wet beriberi disappeared symptoms of dry beriberi developed. In other cases, dry beriberi preceded the wet type. Diarrhea was seldom present or severe during the wet phase of beriberi. Massive spontaneous diuresis often took place.

The clinical symptomatology of the dry beriberi was striking. Burning, hyperesthesias, and paresthesias were exceedingly severe. In some camps hundreds of men would walk the floor during the night because of severe pain. Feet were often soaked in ice water, cooled in the snow, or left exposed during cold nights in attempts to alleviate the pain. The feet were so tender that even the lightest touch provoked severe pain. On one occasion a handkerchief was accidentally dropped on the foot of a sleeping soldier. He immediately awoke and cried out in agony. Often just the vibration caused by the passing of an individual within several feet of a soldier with dry beriberi was sufficient to aggravate the pain.

Symptoms suggestive of scurvy were surprisingly infrequent. Fifteen per cent of the subjects gave a history of bleeding gums. In none of these was the bleeding severe. Petechiae were found in only one subject. On examination this individual was found to have a thrombocytopenia. A history of ecchymosis was obtained from only one person in addition to the individual with thrombocytopenic purpura.

Following the intake of a high caloric diet when they were first liberated, in many individuals glossitis, stomatitis, and edema reappeared or became more pronounced.

A history of blood loss was not a prominent feature in these individuals. Six gave a history of bleeding hemorrhoids. In one of these the bleeding was extremely severe. The one individual with thrombocytopenic purpura gave a history of melena. Eleven individuals gave a history of bleeding gums. In each of these the amount of blood lost was extremely small.

DIET

The diet consisted for the most part of rice (white, brown, or red) and "green" soup. The rice was sometimes polished, other times not and was issued in amounts varying from 300 to 700 Gm. per man per day. If a prisoner was not working, or was sick or hospitalized, he was given only 300 to 500 Gm. per day. If working he was given from 500 to 700 grams. In most cases the rice contained rat feces, pebbles, and worms. The most distracting of the three contaminants were the pebbles. Many times whole barley, corn, or soybeans were mixed with the rice. The "green" soup consisted mainly of water to which small amounts of the tops of green vegetables, grass, or green leaves were added. The amount of soybeans available varied from time to time and from camp to camp. To many individuals the soybeans were unpalatable and even under the severest conditions of starvation were refused because of the nausea, vomiting, and diarrhea which resulted from their ingestion. It may be significant, however, that when soybeans were consumed pellagra was not prevalent. The only meat received consisted of a small piece, about 50 to 100 Gm., once or at the most twice a month. During the earlier phases of the war a larger and sometimes more frequent portion was served. During the latter part of the war, in many camps at least, no meat was served. Fish of poor quality was issued one or two times a month at the most. The portions were always small. The only vegetables received were dicon, a Japanese vegetable resembling a combination between a radish and a turnip, carrots, squash, and potatoes. These were served very infrequently. Fresh fruit was not seen. Men resorted to eating snakes, rats, cats, dogs, lizards, frogs, grasshoppers, and snails when these were available, which was infrequent. When animals such as cats and dogs were obtained, not only muscle, but also organs, intestines, eyes, brain, and other portions were eaten. Grasshoppers, if they could be obtained, were boiled to make a soup. For variation they were sometimes roasted. In many cases men received no food for a period of weeks, sometimes only several meals a week, but generally they ate two or three times a day. In all cases the diet was grossly inadequate both in quality and quantity. Food was obtained from outside sources when possible; this opportunity was rare. Men fought over wormy apple cores discovered by the roadside. Red Cross packages were rarely distributed.

PRIOR TREATMENT

The seventy-five subjects had been on a wholesome American diet for from four to ten weeks prior to this study. All had received vitamin supplements either orally or parenterally. Many had received plasma transfusions.

and three had been given whole blood transfusions. Sixteen of our subjects had received from one to twenty liver injections. Ten gave a history of having been treated with iron.

PHYSICAL CONDITION AT THE TIME OF THE SURVEY

At the time of examination all of the men had been released for from two to eight weeks and had been on an adequate diet of from four to ten weeks. The average weight gain for the group since release was 38 pounds. In general, the individuals appeared to be in good condition. About 80 per cent had regained their normal weight. Two characteristic features were present, however; they were a protuberant abdomen and an abnormal distribution of fat. This was present principally over the pelvic girdle and abdomen and was conspicuously absent from the extremities. Twelve individuals still had definite peripheral edema. In thirteen the liver was palpable. Two had a mild glossitis, two an indefinite cheilosis, one follicular hyperkeratosis, one pellagrous dermatitis, and two had residual evidence of optic neuritis. A few individuals had residual evidence of peripheral polyneuritis. One individual had roentgenologic evidence of active pulmonary tuberculosis. This was the only individual in whom an active infection of any nature other than intestinal was demonstrated.

The stools in fifty-seven subjects were examined for parasites. The results were as follows: negative, 25; *Ascaris lumbricoides*, 47; *Necator americanus*, 32; *Endolimax nana*, 14; *Endamoeba coli*, 14; *Endamoeba histolytica*, 5; *Strongyloides stercoralis*, 5; *Trichuris trichiura*, 2; other, 5. In many instances multiple intestinal infestation was present.

In no subject in this group were malarial parasites found in the blood. At the time of the examination all individuals were free of clinical symptoms of malaria, and none gave a recent history of malaria.

HEMATOLOGIC DATA

Incidence of Anemia.—Hemoglobin values below 14 Gm. per cent were found in 52 per cent of the first 1,500 patients seen (Fig. 1). Hemoglobin values less than 14 Gm. per cent were found in 62 per cent of 193 examinees seen during the first week of the survey. In each successive week there was a tendency for the incidence of anemia to diminish (Fig. 2). By the sixth week the incidence had diministed to 35 per cent.

Severity of the Anemia.—The anemia was either mild (from 11 to 14 Gm. per cent hemoglobin) or moderate in severity (from 8 to 11 Gm. per cent hemoglobin) (Fig. 1). Only one case of severe anemia was observed. In Fig. 3 the severity of anemia as demonstrated by hematocrit determinations is presented. The values fell between 27 and 42 c.c. per 100 c.c. In the majority of cases the volume of packed red cells fell between 34 and 40.

Classification of the Anemia.—In 73 per cent of the seventy-five cases studied the mean corpuscular volume was greater than 92 cubic microns, indicating a macrocytic anemia (Table II). In most cases the macrocytosis was mild (from

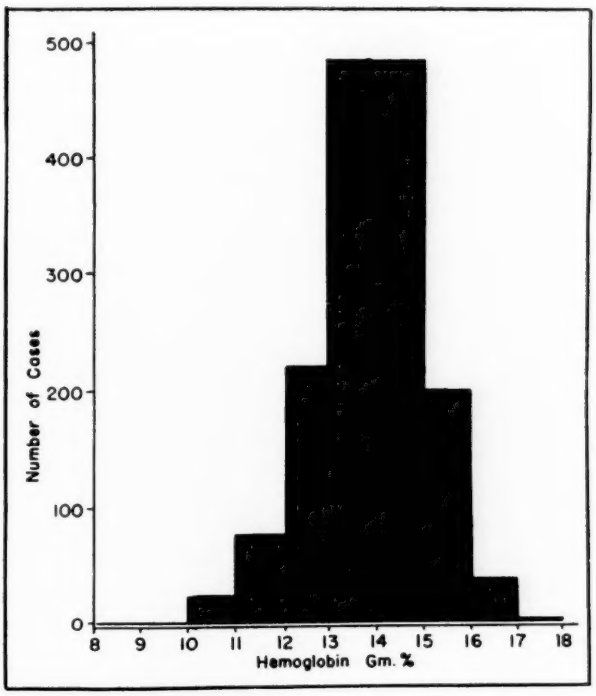

Fig. 1.—Hemoglobin values in the first 1,500 individuals observed.

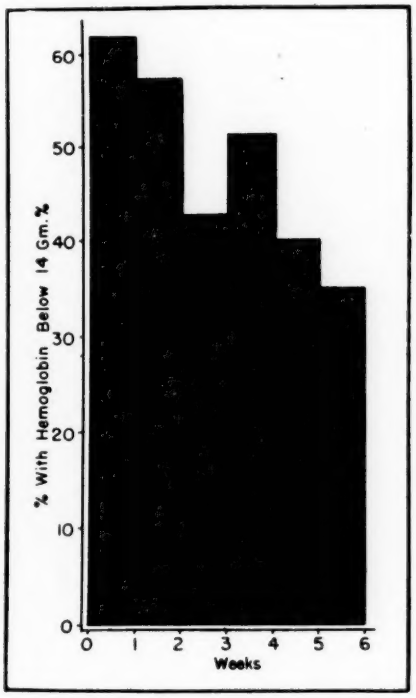

Fig. 2.—The incidence of anemia during each successive week of the survey (3,000 cases).

94 to 104 cubic microns). In twenty cases the mean corpuscular volume was greater than 104 cubic microns, the greatest being 125 cubic microns (Fig. 4). The mean corpuscular hemoglobin for this group was either in the upper range of normal or slightly increased (from 33 to 38 micromicrograms). In two-thirds of the patients studied the mean corpuscular hemoglobin concentration (Fig. 5) fell within the normal range (from 32 to 36 per cent). In the remaining third values were reduced to between 26 and 32 per cent. In the eight patients in whom the mean corpuscular hemoglobin concentration was below 30 per cent, three gave a history of bleeding hemorrhoids, and hookworm infestation was present in two.

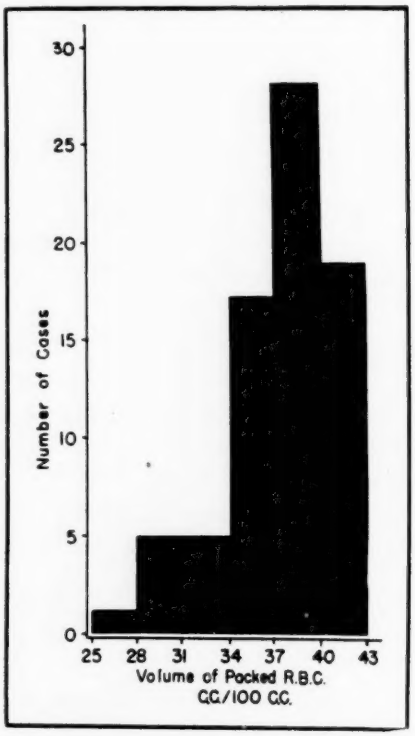

Fig. 3.—The severity of the anemia as measured by hematocrit determinations in the seventy-five subjects studied in detail.

One of the men with a mild macrocytic anemia (mean corpuscular volume, 103; volume of packed red blood cells, 30.6) presented an extensive purpura. The platelet count was reduced to 25,000, the bleeding time was markedly prolonged, and the reticulocytes were increased. He gave a history of blood loss.

In seventeen cases (23 per cent) the anemia was normocytic. Three cases of microcytic anemia (4 per cent) were found. There was a history of definite blood loss in two of these. In the third no history of blood loss was elicited, but the stools were infested with hookworms. In all three cases both

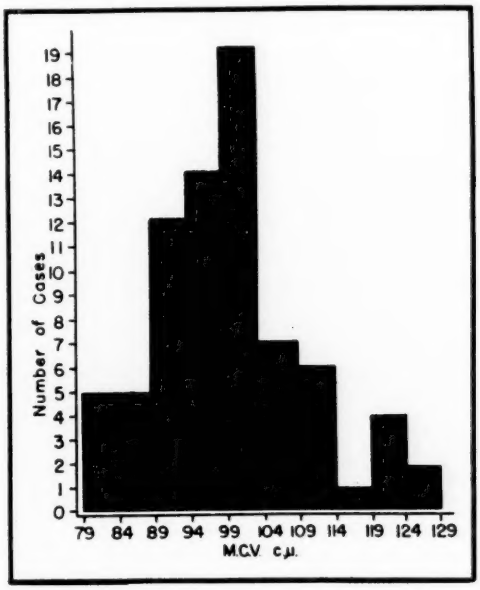

Fig. 4.—The distribution of the mean corpuscular volumes in the seventy-five subjects.

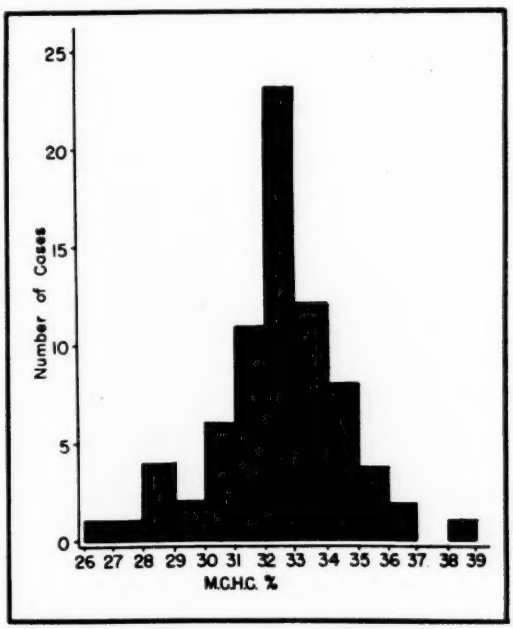

Fig. 5.—The distribution of the mean corpuscular hemoglobin concentrations in the seventy-five subjects.

the microcytosis and the hypochromia were mild. The anemia in all three instances was only mild or moderate in severity. One of these men had previously received iron.

Description of the Blood Films.—In the macrocytic group the cells appeared large and well filled with hemoglobin. A mild hypochromia was observed in a few cases only. Macrocytes were common, in some films numerous. Accompanying the macrocytosis was slight anisocytosis and moderate polychromatophilia. In a few cases the polychromatophilia was extremely marked. Nucleated red cells were occasionally seen, especially in the earlier cases studied and in those with marked macrocytosis. Stippling was seen in only one film

TABLE II. CLASSIFICATION OF ANEMIA ACCORDING TO MEAN CORPUSCULAR VOLUME

TYPE OF ANEMIA	NUMBER OF CASES	PER CENT
Macrocytic	55	73
Normocytic	17	23
Microcytic	3	4
Total	75	100

In the normocytic group the foregoing changes were seen but were less frequent. Numerous macrocytes were observed in three films, and an occasional macrocyte was found in three additional cases. In these instances anisocytosis and occasionally poikilocytosis were present. Three of the subjects presented polychromatophilia. Hypochromia was noted in three films.

Reticulocytes.—Reticulocyte counts were done in twenty of the subjects. In ten of these the percentage of reticulocytes was between 0.4 and 2.0. In the remaining ten the reticulocyte count was between 2.0 and 6.0 per cent. In only three of these subjects was the percentage 4.0 or above.

Icterus Index.—The icterus index was within the normal range in all except one case. This exception was the man with thrombocytopenic purpura.

Leucocytes.—White blood cell counts were done on fifty-three of the subjects. In nineteen the white cell count was between 5 and 10 thousand; in thirty-one the count ranged between 10 and 20 thousand (Fig. 6). In three individuals a leucocytosis greater than 20,000 was noted. In each of these a marked eosinophilia was present. No instances of leucopenia were observed.

Differential counts were not remarkable except for frequent and marked eosinophilia (Fig. 7) and an occasional mild increase in the lymphocytes or monocytes at the expense of the neutrophiles. Neutropenia was not observed. Abnormal or young white cells were not seen. Multinucleated neutrophiles were found in only two films.

Serum Iron.—Serum iron determinations were made on thirty-eight individuals. In seventeen of these (45 per cent) the value fell within the normal range (from 90 to 150 micrograms per cent) as determined by us in normal healthy young men. In twenty-one (55 per cent) the serum iron fell below the normal range. In seven cases the hypoferremia was extremely severe

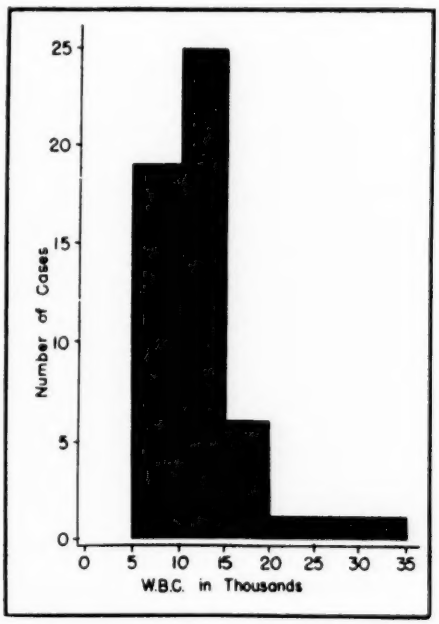

Fig. 6.—The distribution of the white cell counts in the seventy-five subjects.

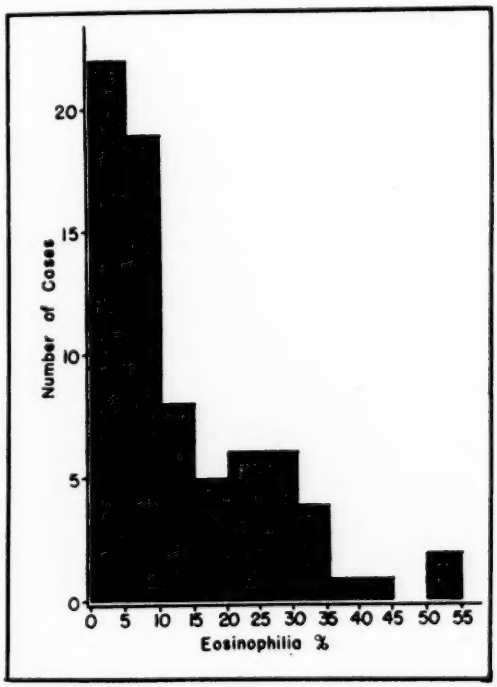

Fig. 7.—The degree of eosinophilia in the seventy-five subjects.

TABLE III. SUMMARY OF SERUM IRON AND COPPER DETERMINATIONS

CONDITION	NUMBER OF CASES	PER CENT
Normal serum iron	17	45
Hypoferremia	21	55
Hyperferremia	0	0
Normal serum copper	19	50
Hypocupremia	0	0
Hypercupremia	19	50

(below 50 micrograms per cent). The lowest value obtained was 16 micrograms per cent. The results are summarized in Table III. No case of hyperferremia was found.

Serum iron determinations done on five subjects with a mean corpuscular hemoglobin concentration of less than 30 per cent revealed a marked hypoferremia in each case.

Serum Copper.—Serum copper determinations weere made on thirty-eight individuals. In nineteen of these (50 per cent) the values fell within the normal range (92 to 140 micrograms per cent). In the others the values were elevated. The highest value obtained was 215 micrograms per cent. The results are summarized in Table III. No case of hypocupremia was found.

DISCUSSION

Although the anemias seen were usually mild or moderate in degree, there is reason to believe that more severe anemia had been present at one time. During each succeeding week of the survey the incidence of anemia diminished, indicating progressive recovery. All of the men had received a wholesome diet fortified with vitamins for a period of from four to ten weeks prior to this study. Verbal reports from medical officers who examined the soldiers immediately following liberation indicate that in most cases the hemoglobin was below 50 per cent of normal at that time. It must be kept in mind that many individuals did not survive and that the most severely ill ones were not evacuated and consequently were not included in this study.

Most of the individuals were able to give a very concise and definite history. In most cases the history was probably quite reliable. These men had had a very intimate and extensive experience with the various signs and symptoms of nutritional deficiency.

Normal serum iron and copper values were found in about half of the individuals studied. Hypoferremia and hypercupremia were found in the other half. Hypoferremia is usually a manifestation of iron deficiency or infection. In general, there is a reciprocal relationship between iron and copper in the serum; that is, when the serum iron is low, the serum copper is elevated. This relationship has held in these studies. However, in experimental iron plus copper deficiency in animals, there is a depression of both the serum iron and the serum copper values. Thus in the present study no evidence of copper deficiency was found. Such a condition (hypocupremia) has never been demonstrated in human beings.

The hypoferremia accompanied by hypercupremia, together with the finding of a slight hypochromia in one-third of the subjects, is suggestive of a mild iron deficiency. It is to be noted, however, that similar changes in the iron and copper content of the serum are found in cases of anemia due to infection.[3] There was no correlation between the degree of hypoferremia or hypercupremia and the degree of anemia.

This study sheds no light on the etiology of the anemia. Under the existing conditions it was not possible to study its pathogenesis. The response to specific factors could not be tested. Therefore, no conclusions can be drawn, and a discussion of the etiology must be speculative. Certain general statements can be made, however. The possible etiologic factors were blood loss, malaria, infections, parasitic infestation, and nutritional deficiency.

It is unlikely that blood loss was a factor in most of the cases. A history of blood loss was obtained in only seven patients. In six of these the blood loss was due to hemorrhoids and was extremely mild. Only one of these men gave a history of recent blood loss. Furthermore, in a great majority of cases, the anemia was macrocytic or normocytic. In the three cases of microcytic anemia it is likely that the anemia was due to blood loss in addition to an inadequate intake of iron.

Although 76 per cent of the group studied gave a history of malaria, in none was the infection recent. Malaria parasites were not found in the blood of any of our subjects. Splenomegaly was not found in any of the men, nor was the icterus index elevated. Thus, it is very unlikely that the anemia was due to malaria.

Infections, local or systemic, were not demonstrated in any of the individuals studied, excepting the one with pulmonary tuberculosis. At the time seen all individuals were afebrile and ambulatory. Although a few gave a history of pneumonia, none had had pneumonia recently. No adequate explanation for the leucocytosis is available. Whether the parasitic infestation was the cause cannot be stated. There was no correlation between the degree of leucocytosis and the degree of anemia. In general, there was no correlation between the degree of eosinophilia and the degree of leucocytosis, although in three individuals with a leucocytosis greater than 20,000 a marked eosinophilia was present (52, 55, and 21 per cent, respectively).

Intestinal parasites were demonstrated in about 75 per cent of the individuals studied. What part parasitic infestation played in the production of the anemia cannot be stated. In 25 per cent of the anemic individuals no parasites were demonstrated. Hookworm infestation was present in 32 per cent. If this parasite was the cause of the anemia, other factors probably were also concerned, since in most cases the anemia was macrocytic. Hookworm anemia is generally hypochromic and microcytic in type. As stated previously, there was no correlation between the degree of eosinophilia and the degree of anemia.

Since all but one patient had received an exceedingly inadequate diet for from thirty-nine to forty-six months and many at one time presented evi-

dence of beriberi and pellagra, it is very possible, even likely, that a deficiency of dietary factors essential for erythropoiesis existed. In some cases (about 50 per cent) this was probably complicated by malabsorption as a result of diarrhea. Since most of the individuals presented a macrocytic anemia, the anemia resembles the so-called nutritional macrocytic anemia so frequently encountered in the Orient in people who are known to subsist on a diet similar to the one on which the American prisoners of war had been placed.[6-12] One difference is apparent. Leucopenia has been frequently observed in nutritional macrocytic anemia. This was not observed in the individuals in this study. Serum iron has been reported to be normal or low in nutritional macrocytic anemia, as many cases are complicated by a mild dietary deficiency of iron.[13] It has been demonstrated that nutritional macrocytic anemia responds to a factor, or factors, present in marmite, an autolyzed yeast preparation, and campolon, a crude liver extract.[6, 7] Whether or not a response to purified liver extract (anahemin) is obtainable is in dispute.[13] Wills and Evans[6] in a series of studies concluded that the active substance is separate and distinct from the extrinsic factor of Castle. Moore and his co-workers[13] have found that the nutritional macrocytic anemia occurring in nutritionally deficient individuals in this country responds to substances containing Castle's extrinsic factor.

SUMMARY AND CONCLUSIONS

A hematologic survey was made on seventy-five repatriated American prisoners of war selected from a series of 3,000. These individuals had been on a grossly inadequate diet consisting mainly of rice for an average of 40.1 months. The average weight loss per individual was 37 pounds. A history of nutritional deficiency as characterized by cheilosis, glossitis, stomatitis, dermatitis, and diarrhea was obtained in from 50 to 70 per cent. A history suggesting wet beriberi was obtained in 77 per cent and a history of dry beriberi in about 50 per cent.

Anemia was observed in 52 per cent of the first 1,500 men examined. The incidence of anemia diminished appreciably as successive groups were studied. Six weeks after the study was commenced, anemia was found in only 35 per cent. In most cases the anemia was only mild or moderate in degree.

Among the seventy-five anemic individuals studied in detail, the anemia was macrocytic in 73 per cent, normocytic in 23 per cent, and microcytic in 4 per cent. Thrombocytopenia was present in one patient. In this individual there was a mild elevation of the icterus index. In the remaining cases the icterus index was normal. A mild reticulocytosis and a leucocytosis (sometimes marked) were frequently encountered. Differential counts were not remarkable, except for the frequent occurrence of an eosinophilia and an occasional increase in lymphocytes or monocytes. Blood films showed numerous macrocytes, anisocytosis, polychromatophilia, and an occasional nucleated red blood cell. In approximately half of the individuals studied normal

serum iron and copper values were found. The other half were characterized by hypoferremia and hypercupremia.

The etiologic factors concerned in the production of these anemias were not studied. It is suggested, however, that most of the men examined probably presented the recovery phase of severe nutritional macrocytic anemia. The role played by intestinal parasitic infestations in the etiology of the anemia could not be determined. Malaria, blood loss, and infections were probably not important contributing factors in most of the cases. Iron deficiency may have been a contributing factor in a few. The three cases of microcytic anemia were most likely due to blood loss.

REFERENCES

1. Wintrobe, M. M.: Clinical Hematology, ed. 1, Philadelphia, 1942, Lea and Febiger.
2. Kitzes, G., Elvehjem, C. A., and Schuette, H. A.: The Determination of Blood Plasma Iron, J. Biol. Chem. **155:** 653, 1944.
3. Cartwright, G. E., Lauritsen, M. A., Jones, P. J., Merrill, I. M., and Wintrobe, M. M.: The Anemia of Infection. I. Hypoferremia, Hypercupremia and Alterations in Porphyrin Metabolism in Patients, J. Clin. Investigation **25:** 65, 1946.
4. Powell, J. F.: Serum-Iron in Health and Disease, Quart. J. Med. **13:** 19, 1944.
5. Cartwright, G. E., Jones, P. J., and Wintrobe, M. M.: A Method for the Determination of Copper in Blood Serum, J. Biol. Chem. **160:** 593, 1945.
6. Wills, L., and Evans, B. D. F.: Tropical Macrocytic Anemia: Its Relation to Pernicious Anemia, Lancet **2:** 416, 1938.
7. Wills, L.: Treatment of "Pernicious Anemia of Pregnancy" and "Tropical Anemia," Brit. M. J. **1:** 1059, 1931.
8. Wills, L.: Studies in Pernicious Anemia of Pregnancy. VI. Tropical Macrocytic Anemia as a Deficiency Disease, With Special Reference to the Vitamin B Complex, Indian J. M. Research **21:** 669, 1934.
9. Napier, L. E.: The Etiology of Tropical Macrocytic Anemia, Indian M. Gaz. **74:** 1, 1939.
10. Napier, L. E., Das Gupta, C. R., Chaudhuri, R. N., Sen, G. N., Rai Chaudhuri, M. N., Sen Gupta, P. C., and Majumdar, D. N.: Anahaemin in Tropical Macrocytic Anaemia, Indian M. Gaz. **73:** 385, 1938.
11. Foy, H., and Kondi, A.: Response of Nutritional Macrocytic Anaemia to Anahaemin, Lancet **2:** 360, 1939.
12. Fairley, N. H., Bromfield, R. J., Foy, H., and Kondi, A.: Nutritional Macrocytic Anaemia in Macedonia; a preliminary report, Tr. Roy. Soc. Trop. Med. & Hyg. **32:** 132, 1938.
13. Moore, C. V., Vilter, R., Minnich, V., and Spies, T. D.: Nutritional Macrocytic Anemia in Patients With Pellagra or Deficiency of the Vitamin B Complex, J. LAB. AND CLIN. MED. **29:** 1226, 1944.

ABSORPTION AND EXCRETION OF SULFATHIADIAZOLE AND SULFAMETHYLTHIADIAZOLE AFTER ORAL ADMINISTRATION IN HUMAN BEINGS

MANSON MEADS, M.D., AND MAXWELL FINLAND, M.D.
BOSTON, MASS.

THERE is still some interest in the development of nontoxic chemotherapeutic agents which are effective in the treatment of intestinal and urinary tract infections. White and co-workers[1] have recently reported a study of the antibacterial activity of some sulfacarboxythiazoles and of sulfathiadiazole against the dysentery group of organisms. The results which they obtained with two of these compounds, sulfacarboxythiazole (2-sulfanilamido-5-carboxythiazole) and sulfathiadiazole (2-sulfanilamido-1,3,4-thiadiazole), were of interest and seemed to warrant further study.

Sulfacarboxythiazole was found to be about as active as sulfaguanidine against twelve dysentery strains in vitro and against coliform organisms in mice. It is highly soluble at the acid pH's which may be expected in the intestinal tract, is very poorly absorbed, and presumably owes its activity to release of sulfathiazole. It is similar, in these respects, to phthalylsulfathiazole (sulfathalidine). In a limited study of sulfacarboxythiazole in human beings,[2] it was found to be very poorly absorbed after oral administration, produced no toxic symptoms, and appeared to be effective in a few cases of dysentery. This compound was also studied by Poth and Ross[3] who found it and some related compounds to be of relatively low toxicity and high activity in the intestinal tract of the dog.

The synthesis and physical properties of sulfathiadiazole have been reported from two separate laboratories.[4, 5] Its activity in vitro was found to be similar to that of sulfanilamide,[5, 6] and in mice it was active against pneumococcal but not against streptococcal infections.[4] Frisk[6] also studied the pharmacology and biologic activity of the 5-methyl and 5-ethyl derivatives of sulfathiadiazole and found them to be rapidly and almost completely absorbed, nontoxic in therapeutic doses, highly soluble, rapidly and completely excreted in the urine, minimally acetylated, and biologically about as active as sulfathiazole against pneumococcus types 1 and 3 and *Escherichia coli* in vitro and against type 1 pneumococcal infection in mice. Frisk's monograph did not include studies on the absorption and excretion or on the in vivo activity of sulfathiadiazole, presumably because of the relatively low activity which he found in vitro.

From the comparative studies of White and co-workers,[1] however, sulfathiadiazole appeared to possess interesting possibilities for use against dysentery. As compared with sulfaguanidine it was ten times as soluble at pH 6.5, from four to eight times as active against several dysentery strains in vitro, and more

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.
Received for publication, June 10, 1946.

active against coliform bacteria in mice. This compound did not appear to owe its activity to a decomposition product as did sulfacarboxythiazole or sulfathalidine. In preliminary pharmacologic studies, Robinson[7] found high concentrations in the intestinal contents, moderately low concentrations in the blood, and relatively high, but due to its solubility probably safe, concentrations in the urine. Studies of the absorption and excretion of this compound after oral administration in human beings, therefore, seemed warranted. Such studies are reported in this paper together with some comparative observations on the 5-methyl derivative.

MATERIALS AND METHODS

The subjects were normal laboratory workers or convalescent men patients who had not received sulfonamide drugs recently and had no evidence of cardiac or renal insufficiency. They all weighed between 66 and 77 kilograms. The first part of the study was carried out on two subjects and was essentially a repetition with sulfathiadiazole of the observations previously reported with sulfacarboxythiazole.[2] In the second part, a single 5 Gm. dose of sulfathiadiazole was given to each of five subjects, and the 5-methyl derivative* was given to three subjects. Except when otherwise noted, the daily fluid intake was about 2,500 to 3,000 c.c., and no alkalies were given. All of the single doses were given at 9:00 A.M. All of the urine was collected at two-hour intervals during the first twelve hours and at twelve-hour intervals during the next thirty-six hours. Bloods for levels were obtained at these times and also one-half and one hour after the dose, but the fecal excretion was not studied. The urines passed at odd intervals also were studied in several other subjects while receiving repeated one Gm. doses after an initial four Gm. dose.

RESULTS

Excretion of Sulfathiadiazole After a Single 5 Gm. Oral Dose.—The free and total sulfathiadiazole content of the urines collected in twelve-hour periods and of each of the stools passed by two subjects are given in Table I. Subject G. B., who received no laxatives, excreted 75 per cent of the dose in the urine and 4.5 per cent in the stool. Subject B. S. was given a laxative (cascara sagrada) each morning and evening from the day before the study was begun until it was ended. A total of 60.1 per cent of the dose was recovered from the urine and 8.1 per cent from the feces of subject B. S. Of the total drug recovered from the urines, 83 per cent was excreted in the first twelve hours by G. B. and 93 per cent by B. S. In both subjects only a small percentage of the urinary sulfathiadiazole and a somewhat greater proportion of the fecal drug were in a conjugated form. The significance of the latter is not entirely clear. The blood levels at one and one-half hours were about the same in both subjects. Some drug was still present in the blood of subject G. B. eight hours after the dose was given, but none was demonstrated at that time in the blood of subject B. S.

*The sulfonamides used in these studies were furnished by the Lederle Laboratories, Inc., Pearl River, N. Y. The chemical determinations were carried out by Ellen J. Doyle.

TABLE I. SULFATHIADIAZOLE IN URINE AND FECES AFTER SINGLE ORAL DOSE OF 5 GM.

SUBJECT G. B.						SUBJECT B. S.					
URINE			FECES			URINE			FECES		
HOUR AFTER DOSE	FREE MG.	TOTAL MG.	HOUR AFTER DOSE	FREE MG.	TOTAL MG.	HOUR AFTER DOSE	FREE MG.	TOTAL MG.	HOUR AFTER DOSE	FREE MG.	TOTAL MG.
0 to 12	2,960	3,120	0 to 30	113	140	0 to 12	2,575	2,810	0 to 8	73	110
12 to 24	225	366	30 to 55	70	85	12 to 24	85	160	8 to 10	150	200
24 to 36	113	191	55 to 81	0	0	24 to 36	16	20	10 to 32	80	95
36 to 48	17	26	81 to 104	0	0	36 to 48	11	13	32 to 56	0	0
48 to 60	11	20				48 to 60	0	0			
60 to 72	9	16				60 to 72	0	0			
72 to 84	0	10									
0 to 84	3,335	3,749	0 to 104	183	225	0 to 72	2,687	3,003	0 to 56	303	405
Per cent conjugated		11.0	Per cent conjugated		18.7	Per cent conjugated		10.5	Per cent conjugated		25.2
Per cent recovered		75.0	Per cent recovered		4.5	Per cent recovered		60.1	Per cent recovered		8.1
Blood (mg. %):						Blood (mg. %):					
At 1½ hr. = 3.4 free; 4.1 total						At 1½ hr. = 3.6 free; 4.2 total					
At 8 hr. = Trace free; 1.5 total						At 8 and 24 hr. = None					
At 24 hr. = None											

TABLE II. SULFATHIADIAZOLE IN BLOOD, URINE, AND FECES AFTER ORAL ADMINISTRATION OF 4 GM. FOLLOWED BY 1 GM. EVERY FOUR HOURS FOR TOTAL OF 25 GM. IN EIGHTY-FOUR HOURS

SUBJECT G. B.						SUBJECT B. S.					
URINE			FECES			URINE			FECES		
HOUR AFTER FIRST DOSE	FREE MG.	TOTAL MG.	HOUR AFTER FIRST DOSE	FREE MG.	TOTAL MG.	HOUR AFTER FIRST DOSE	FREE MG.	TOTAL MG.	HOUR AFTER FIRST DOSE	FREE MG.	TOTAL MG.
0 to 12	2,100	2,265	0 to 58	1,060	1,150	0 to 12	1,970	2,063	0 to 9	55	67
12 to 24	1,910	2,000	58 to 106	540	660	12 to 24	2,570	3,240	9 to 36	372	410
24 to 36	1,973	2,200	106 to 155	23	27	24 to 36	1,622	1,935	36 to 54	370	410
36 to 48	1,155	1,520				36 to 48	2,180	2,790	54 to 83	200	215
48 to 60	2,625	2,880	0 to 155	1,623	1,837	48 to 60	340	449	83 to 105	179	196
60 to 72	1,810	2,010				60 to 72	2,150	3,580	105 to 125	53	58
72 to 84	1,633	1,862	Per cent conjugated		11.6	72 to 84	714	828	125 to 137	25	26
84 to 96	1,385	1,783				84 to 96	1,065	1,248	137 to 149	0	0
96 to 108	446	528	Per cent recovered		7.3	96 to 108	61	84			
108 to 120	165	175				108 to 120	41	55	0 to 149	1,254	1,382
120 to 132	119	145	BLOOD (PER 100 C.C.)			120 to 132	0	0			
132 to 144	61	94				132 to 144	0	0	Per cent conjugated		9.3
144 to 156	36	60	1	1.7	2.0	0 to 144	12,713	16,272	Per cent recovered		5.5
156 to 168	5	5	2	3.9	4.4	Per cent conjugated		21.9	BLOOD (PER 100 C.C.)		
168 to 180	0	0	4	2.6	3.0	Per cent recovered		65.1			
			32	1.0	1.7				1	4.9	5.5
0 to 180	15,423	17,527	49	2.0	2.7				2	3.1	4.1
Per cent conjugated		12.0							4	1.2	1.4
Per cent recovered		70.1							32	trace	trace
									49	1.0	1.6

Excretion of Sulfathiadiazole After Repeated Oral Doses.—The same two subjects were later given repeated doses of sulfathiadiazole, and the amounts recovered from urine and feces were determined. In addition, blood levels were determined hourly for four hours after the initial 4 Gm. dose and twice thereafter. The results are shown in Table II. In this instance the total amounts of drug recovered in the urine and feces were quite comparable in the two subjects, most of it being found in the urine. More of the urinary sulfathiadiazole in subject B. S. and less of the fecal drug recovered in both subjects were in the conjugated form. The maximum blood level was obtained two hours after the initial 4 Gm. dose in G. B. and one hour after that dose in B. S., but in both subjects appreciably lower levels were found in the thirty-two and forty-nine hour bloods which were drawn four hours and one hour, respectively, after a 1 Gm. dose.

The concentration of the drug in the different specimens varied depending, of course, on the volume of urine excreted. In Subject G. B. the concentrations ranged from 540 to 240 mg. per cent for the total and from 500 to 182 mg. per cent for the free drug during the time when it was being given. In subject B. S. the range was between 430 and 160 mg. per cent for the total and between 410 and 100 mg. per cent for the free drug. Neither crystals nor red blood cells were found in any of the specimens, but the subjects took fluids liberally (about 3 liters per day).

TABLE III. SULFATHIADIAZOLE IN INDIVIDUAL SPECIMENS OF URINE DURING ADMINISTRATION OF REPEATED ORAL DOSES

SUBJECT	DAY	ESTIMATED FLUID INTAKE (C.C. PER DAY)	PH OF URINE	DRUG IN DIFFERENT SPECIMENS OF VOIDED URINE (MG. %)				PER CENT CONJUGATED	
				MAXIMUM		MINIMUM			
				FREE	TOTAL	FREE	TOTAL	RANGE	AVERAGE
J. S.	1	1,500	5.8 to 7.0	1,030	1,180	610	670	9 to 12	10.8
	2	2,500	5.4 to 5.7	900	910	170	190	4 to 27	10.4
	3 to 4	3,500*	7.2 to 8.8	410	450	70	95	4 to 24	16.5†
L. G.	1	5,000	6.5 to 7.0	415	480	10	10	0 to 14	11.6
	2	900	5.2 to 6.0	500	584	150	190	4 to 24	11.3
	3	2,500	5.2 to 6.5	390	450	240	310	13 to 22	18.5‡
R. L.	1 to 4	3,500	5.3 to 6.8	202	232	64	70	9 to 21	14.2

*Sodium bicarbonate, 6 Gm. followed by 2 Gm., with each dose given on these days.
†Average for the four days was 11.6 per cent.
‡Average for the three days was 13.3 per cent.

Three additional patients were given 4 Gm. each of sulfathiadiazole followed by 1 Gm. every four hours, and all voided specimens were collected during the time when the drug was being administered. The pH of each specimen was tested, and the concentrations of free and total sulfathiadiazole were determined. The results are summarized in Table III in relation to the fluid intake. The expected effect of the fluid intake is clearly shown. Several specimens from Subject J. S. contained more than 1 per cent of drug. The sediments of all the voided specimens in these cases were examined and found to be free of red blood cells and drug crystals.

Rapidity of Absorption and Excretion of Sulfathiadiazole and Sulfamethylthiadiazole After a Single Oral Dose.—Since most of the drug which appeared in the urine after a single 5 Gm. oral dose of sulfathiadiazole was recovered during the first twelve hours, it was of interest to determine in more detail when the maximum absorption and excretion occurred. For that purpose a single 5 Gm. dose was given to five subjects, and the total urine output was collected at two-hour intervals during the first twelve hours and at twelve-hour intervals for the next thirty-six hours. Blood levels were determined at these intervals and also at one-half and one hour. A similar study was carried out with sulfamethylthiadiazole in three subjects, two of whom were also used for the sulfathiadiazole study. The results are shown graphically in Figs. 1* and 2.

For both drugs the rapidity of absorption varied considerably as indicated by the maximum blood levels and the time when they were attained. The maximum concentrations of total sulfathiadiazole in the blood varied from 4.0 to 7.8 mg. per cent and the free drug varied from 3.6 to 6.7 mg. per cent. The corresponding figures for sulfamethylthiadiazole were from 6.5 to 12.0 mg. per cent total and from 6.2 to 10.8 mg. per cent free. These maximums were attained in different subjects from one to four hours after the ingestion of either drug.

TABLE IV. URINARY EXCRETION OF SULFATHIADIAZOLE AND SULFAMETHYLTHIADIAZOLE AFTER SINGLE 5 GM. DOSE

SUBJECT	PERCENTAGE OF TOTAL DRUG EXCRETED IN THE URINE RECOVERED IN THE FIRST				
	2 HOURS	4 HOURS	6 HOURS	10 HOURS	24 HOURS
		Sulfathiadiazole			
J. M.	3	28	58	88	100
E. O.	5	45	69	93	99
R. L.	21	39	70	90	98
M. M.	22	65	89	97	99
B. S.	44	83	91	96	100
		Sulfamethylthiadiazole			
E. O.	8	29	52	90	99
J. B.	5	47	73	95	99
M. M.	50	83	95	99	100

The urinary excretions showed corresponding differences. The maximum excretion occurred in different subjects, either in the first, second, or third two-hour interval. The greatest variations in excretion occurred in the first and second two-hour periods. Practically all of the urinary excretion occurred in the first twenty-four hours, and very little was excreted after ten hours in most of the subjects and after six hours in some of them. This is shown in Table IV. The total amount of drug excreted by each of the subjects and the proportion which was determined as being in the conjugated form are shown in Table V. From 60 to 100 per cent of the administered sulfathiadiazole (average, 83.4 per cent) was recovered in the urine, while the sulfamethylthiadiazole was completely recovered in the urine in each of the three subjects.

The maximum concentrations found in the urine in these subjects are shown, together with other relevant data in Table VI. The maximum concentration of

*The findings in subject J. M. are omitted from this figure because frequent blood levels were not determined.

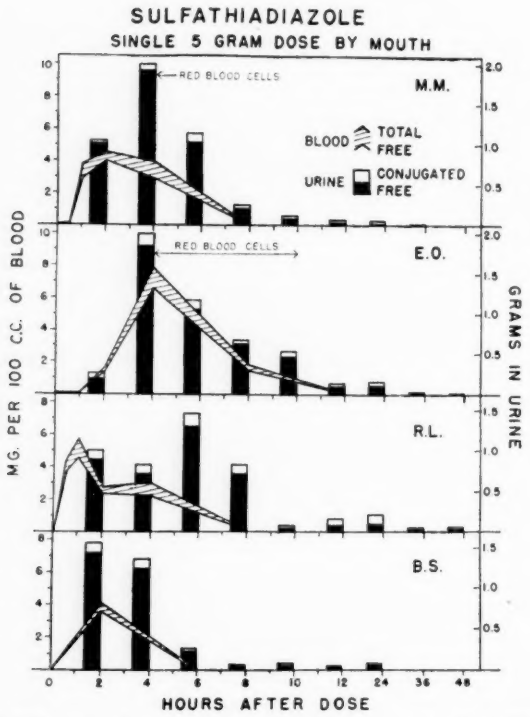

Fig. 1.—Blood levels and urinary excretion of sulfathiadiazole in four subjects each given a single dose of 5 Gm. by mouth.

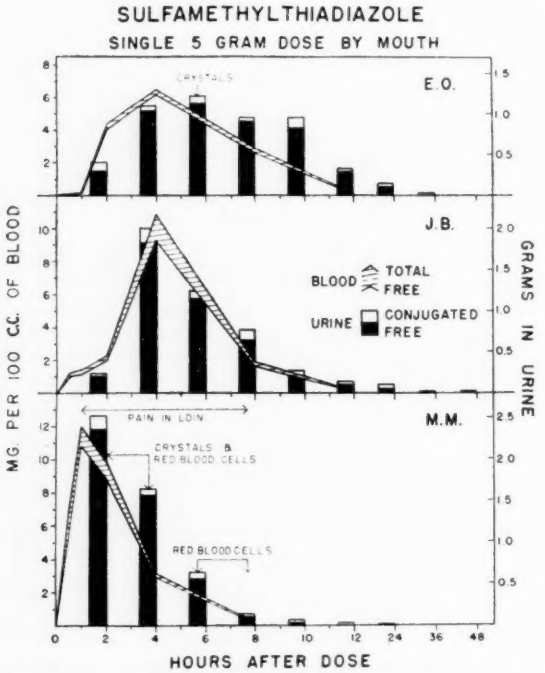

Fig. 2.—Blood levels and urinary excretion of sulfamethylthiadiazole in three subjects each given a single dose of 5 Gm. by mouth.

TABLE V. TOTAL URINARY EXCRETION OF SULFATHIADIAZOLE AND SULFAMETHYLTHIADIAZOLE AFTER SINGLE ORAL 5 GM. DOSE

SUBJECT	GM. IN URINE		PER CENT OF DOSE EXCRETED	PER CENT CONJUGATED
	FREE	TOTAL		
		Sulfathiadiazole		
B. S.	2.687	3.003	60.1	10.5
B. S.	3.172	3.511	70.2	9.6
G. B.	3.335	3.749	75.0	11.0
J. M.	4.142	4.541	90.8	8.8
R. L.	3.955	4.700	94.0	15.8
M. M.	4.392	4.702	94.0	6.6
E. O.	4.515	4.987	99.7	9.5
Average	3.743	4.170	83.4	10.2
		Sulfamethylthiadiazole		
J. B.	4.239	4.805	96.1	11.8
M. M.	4.737	5.046	100.9	6.2
E. O.	4.801	5.264	105.3	8.8
Average	4.592	5.038	100.8	8.9

TABLE VI. MAXIMUM CONCENTRATION OF SULFONAMIDES IN URINE AFTER SINGLE 5 GM. DOSE

SUBJECT	INTERVAL AFTER DOSE (HR.)	VOLUME (C.C.)	PH	MG. PER 100 C.C.		SEDIMENT	
				FREE	TOTAL	CRYSTALS	R.B.C.
			Sulfathiadiazole				
J. M.	2 to 4	385	--	293	300	0	0
E. O.	2 to 4	125	5.5	1,480	1,600	0	Few*
R. L.	2 to 4	65	5.5	1,100	1,270	0	0
M. M.	2 to 4	180	6.0	1,320	1,380	0	Rare
B. S.	2 to 4	290	--	428	472	0	0
			Sulfamethylthiadiazole				
E. O.	4 to 6	90	5.8	1,260	1,360	Many	0
J. B.	2 to 4	150	5.5	1,220	1,360	0	0
M. M.	0 to 2	175	6.0	1,360	1,440	Many	Many†

*There were occasional red blood cells but no crystals in the sediment of later specimens collected up to eight hours.

†Two hours after the dose this subject developed pain in the right loin radiating to the groin and accompanied by nausea. Crystalluria was also present in the two- to four-hour specimen, and microscopic hematuria persisted for four hours longer. The two- to 4-hour specimen consisted of 150 c.c., pH 5.5, and contained 1,060 mg. per cent free and 1,100 mg. per cent total drug.

sulfathiadiazole was found in the two- to four-hour specimen in each instance and varied from 300 to 1,600 mg. per cent. The concentration of the methyl compound reached from 1,360 to 1,440 mg. per cent in the three subjects, but these maximums were found at different intervals after the dose. Only a small percentage of the drug was found in the urine in the conjugated form. These specimens were all acid (pH 5.5 to 6.0).

Distribution Between Plasma and Blood Cells.—The distribution of sulfathiadiazole and sulfamethylthiadiazole between plasma and red blood cells was studied in two subjects at the time of the maximum blood level after a single oral dose. The results are shown in Table VII. The plasmas contained 2.5 and 3.1 times as much free sulfathiadiazole and fifteen and twenty-four times as much free sulfamethylthiadiazole as the red blood corpuscles. All of the conjugated drug was in the plasma, and none was found in the red blood cells.

Untoward Effects.—One patient, subject J. B., developed fever (100° F.), conjunctivitis, and urticaria after forty-eight hours of treatment with sulfamethylthiadiazole given in the usual dosage, namely, 4 Gm. followed by 1 Gm.

TABLE VII. DISTRIBUTION IN PLASMA AND RED BLOOD CELLS

SUBJECT	SULFATHIADIAZOLE		SULFAMETHYLTHIADIAZOLE	
	E. O.	M. M.	E. O.	M. M.
Whole blood (mg. %)				
Free	6.6	4.0	6.2	10.8
Conjugated	1.2	0.5	0.3	1.2
Plasma (mg %)				
Free	8.9	5.8	10.4	17.0
Conjugated	2.2	1.3	1.7	2.8
Hematocrit (%)	43	45	43	45
Red blood cells (mg. %) (calculated)				
Free	3.5	1.9	0.7	0.7
Conjugated	0	0	0	0

every four hours. He had received a single 5 Gm. dose four days previously without untoward effect. Fever and symptoms subsided within a few hours after the last dose. The patient was subsequently given test doses of 2 Gm. of sulfathiazole and sulfadiazine and showed no untoward effects. The only other untoward effects observed are those noted in Table VI. It is of interest, however, that several other subjects had concentrations of more than 1 per cent of sulfathiadiazole in urine that was acid at the time and nevertheless showed no hematuria or crystalluria. There was no nausea noted except during the episode of renal colic in Subject M. M. The sulfamethylthiadiazole crystals recovered from the urine of subjects E. O. and M. M. were probably in the free form as judged by the usual chemical determination. They appeared microscopically as large compact sheaths with irregular and jagged ends resembling broken-off tree trunks.

DISCUSSION

The types of compounds which are considered to be useful in the treatment of enteric infections are those which are highly soluble at the acid pH of the intestinal contents and poorly absorbed, so that high concentrations are maintained in the bowel and very little gets into the blood and urine. If there is good absorption, the excreted drug must be highly soluble at the range of pH that may be expected in the urine. For the treatment of urinary tract infections it is desirable to have compounds which are absorbed and excreted fairly rapidly with a minimum of acetylation and are highly soluble in the range of pH of the urine. Both types of drugs must be active, of course, in their respective media against the pathogenic organisms for which they are used.

Sulfathiadiazole and sulfamethylthiadiazole were both found to be rapidly absorbed and excreted minimally conjugated in blood and urine after oral administration. All but a small percentage of the sulfathiadiazole and practically all of the 5-methyl derivative were recovered from the urine, largely in the free form and mostly within the first few hours after they were ingested. Only from 4.5 to 8.1 per cent of the orally administered sulfathiadiazole were recovered from the feces. High concentrations, often over 1 per cent, were obtained in the urine in the first few hours after a single 5 Gm. dose. In most instances these large amounts of drug remained in solution in the urine, even at pH as low as 5.5. In two subjects, however, sulfamethylthiadiazole crystals appeared

in large numbers in the voided urine after a single 5 Gm. dose, and this was accompanied by renal colic and hematuria in one of them. A few red blood cells without crystals were also present in the urine in two subjects after a dose of sulfathiadiazole, but in every instance the urine contained very high concentrations of drug at the time. These complications occurred within a few hours after a 5 Gm. dose and were not seen during repeated administration of 1 Gm. doses at four-hour intervals.

In the preliminary studies of White and co-workers[1] sulfathiadiazole appeared to have therapeutic possibilities for the treatment of dysentery infections and seemed to be active against coliform organisms in mice. The present studies, however, suggest that, from the point of view of its absorption and excretion, this compound is not particularly suited for enteric infections unless the small amounts remaining in the bowel are very highly active. Such a possibility is suggested by the effectiveness of sulfathiazole and sulfadiazine in the treatment of dysentery in spite of the small amounts recoverable in the feces, and sulfathiadiazole has the added advantage of greater solubility at the pH of the intestinal contents. On the other hand, the high solubility throughout the urinary pH range and the effectiveness against coliform organisms suggest that sulfathiadiazole and its 5-methyl derivative may be useful in urinary tract infections. Doses as large as 5 Gm. at a time, however, should be avoided, but doses of 1 Gm. each can probably be given safely every four hours. More experience in the actual treatment of urinary tract infections is needed before the safety of these compounds and their effectiveness can be determined.

CONCLUSIONS

Sulfathiadiazole given by mouth was rapidly and almost completely absorbed, rapidly eliminated in the urine, and highly soluble in the urinary range of pH. About 90 per cent of the drug recovered from the urine was in the free form. Sulfamethylthiadiazole was more completely absorbed and rapidly eliminated in the urine, and it gave somewhat higher maximum blood levels. Microscopic hematuria and macroscopic crystalluria were observed in two of three subjects after a single oral dose of 5 Gm. of the methyl derivative. The findings suggest that sulfathiadiazole deserves a trial in the treatment of urinary tract infections but that large individual doses should be avoided.

REFERENCES

1. White, H. J., and co-workers: Sulfonamides for Bacillary Dysentery. I. The Antibacterial Activity of Sulfacarboxythiazoles and Sulfathiadiazole, J. Pharmacol. & Exper. Therap. **85**: 247-257, 1945.
2. Harris, H. W., and Finland, M.: Carboxythiazole (2-Sulfanilamido-5-carboxythiazole) in Blood, Urine and Feces After Oral Administration in Humans, Proc. Soc. Exper. Biol. & Med. **58**: 116-119, 1945.
3. Poth, E. J., and Ross, C. A.: Bacteriostatic Properties of Sulfanilamide and Some of Its Derivatives. III. The Carboxy-Sulfathiazoles as Chemotherapeutic Agents Active in the Gastrointestinal Tract, J. Lab. & Clin. Med. **30**: 843-849, 1945.
4. Roblin, R. O., Jr., Williams, J. H., Winnek, P. S., and English, J. P.: Chemotherapy. II Some Sulfanilamido Heterocycles, J. Am. Chem. Soc. **62**: 2002-2005, 1940.
5. Vonkennel, J., Kimmig, J., and Korth, B.: Versuche und Untersuchungen mit neuen Sulfonamiden, Ztschr. f. klin. Med. **138**: 695-743, 1940.
6. Frisk, A. R.: Sulfanilamide Derivatives: Chemotherapeutic Evaluation of N^1-Substituted Sulfanilamides, Acta med. Scandinav., Suppl. p. 142, 1943.
7. Robinson, E. J.: Unpublished data quoted by White and co-workers.[1]

THE QUANTITATIVE DETERMINATION OF THE THYMOL TURBIDITY REACTION OF SERUM

ALLYN B. LEY, M.D., JESSICA H. LEWIS, M.D., AND
CHARLES S. DAVIDSON, M.D., C.M.
BOSTON, MASS.

THE thymol turbidity reaction of serum as described by Maclagan[1, 2] has been studied, especially in liver disease, by several observers.[3, 4] Shank and Hoagland[5] have adapted the reaction to the Coleman spectrophotometer, using barium sulfate suspensions as standards.

The determination of Maclagan units of turbidity by the visual comparator block is not precise enough for the statistical analysis of the results. In this paper is reported a modification of the Maclagan method which appears to offer a more quantitative approach to the determination of thymol turbidity as a means of evaluating the presence of liver disease.

METHODS

The entire reaction with serum, as well as the formation of the standard barium sulfate suspensions, was carried out in Evelyn tubes. A 660 μ filter was used in an Evelyn colorimeter. Galvanometer readings G were changed to L values (2 – Log G). Distilled water was used for the 100 setting on the colorimeter rather than the thymol buffer, as the latter tends to become turbid upon standing. Slight turbidity of the thymol buffer clears when it is added to the serum, but in general cloudy buffer should not be used.

Standard Barium Sulfate Curve.—A barium sulfate suspension was prepared as described by Shank and Hoagland,[5] using 3 c.c. of 1 per cent barium chloride diluted to 100 c.c. with 0.2 N sulfuric acid. Serial dilutions of the standard were made with 0.2 N sulfuric acid, and the resulting turbidity was measured in the Evelyn photoelectric colorimeter. Seven serial dilutions of the barium sulfate suspension were made, each one five times. The mean value for each dilution was obtained and a regression equation derived from the data. The equation is: $Y = 20.81X - 0.0271$, where Y = c.c. $BaSO_4$ suspension, and X = L value (2 – Log G). The values obtained are shown in Table I. The curve drawn from these values is a straight line. The approximate Maclagan units corresponding to the dilutions of the $BaSO_4$ standard are given in Table I.

Procedure.—The reaction was carried out as follows: Into an Evelyn tube were placed, in succession, 0.1 c.c. of serum and 6.0 c.c. of thymol-barbiturate buffer prepared according to Maclagan.[2] The contents were well mixed and kept at room temperature for thirty minutes; galvanometer readings were then obtained using distilled water as the 100 setting.

From the Thorndike Memorial Laboratory, the Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.
Received for publication, June 17, 1946.

TABLE I. RELATION BETWEEN VOLUME OF BARIUM SULFATE SUSPENSION, EVELYN READINGS, AND MACLAGAN UNITS

$BaSO_4$ SUSPENSION (C.C.)	0.2 N H_2SO_4 (C.C.)	EVELYN GALVANOMETER READING	L VALUE (2-LOG G)	APPROXIMATE MACLAGAN UNITS
0.5	5.5	94[2]	0.0246	2 ½
1.0	5.0	89[0]	0.0505	5
2.0	4.0	79[3]	0.0982	10 ½
3.0	3.0	71[2]	0.1457	16
4.0	2.0	64[0]	0.1936	22
5.0	1.0	57[0]	0.2441	28
6.0	0.0	31[1]	0.2903	34

The galvanometer readings G were changed to L values (2 – Log G), and the corresponding cubic centimeters of barium sulfate suspension, obtained from the equation by calculation or from the curve, were taken as indicating the degree of equivalence of thymol turbidity.

RESULTS

Normal.—Serum was obtained from 105 apparently healthy young men without a history of gastrointestinal disturbance or jaundice within three months. The serum bilirubin concentrations[6] were normal in all.

The thymol turbidity of these men varied from 0.16 c.c. to 2.19 c.c. of barium sulfate (Table II). The mean was 0.63 c.c. with a standard deviation $\left(\text{S.D.} = \sqrt{\frac{\Sigma\, d^2}{n\text{-}1}} \right)$ of 0.35 cubic centimeter. If the the mean normal plus three times the standard deviation is taken as the upper limit of normal variation, this would give normal limits of thymol turbidity as equivalent to from 0 to 1.68 c.c. of the barium sulfate suspension. In our normal group two sera had values above this.

Cirrhosis of the Liver.—Sixty-three thymol turbidity determinations were made on twenty-three individuals with cirrhosis of the liver (Laënnec). Twelve of the cases were arbitrarily classified, without reference to the thymol turbidity, as mild and eleven as severe. The results are shown in Table II. Eighty-one per cent of the determinations in all cirrhosis were elevated above 1.68 c.c., while in advanced cirrhosis 92 per cent were likewise above normal.

TABLE II. THYMOL TURBIDITY REACTION OF NORMAL SERA CONTRASTED WITH REACTION IN LAËNNEC'S CIRRHOSIS AND ILLNESSES WITHOUT EVIDENCE OF LIVER DISEASE

	DISTRIBUTION IN PERCENTAGE				
			LAËNNEC'S CIRRHOSIS		
THYMOL TURBIDITY (C.C. $BaSO_4$)	NORMAL	ILLNESSES WITHOUT EVIDENCE OF LIVER DISEASE	ALL	MILD	SEVERE
Number of determinations	105	27	63	26	37
< 1	91	33	8	15	3
1.0 to 1.68	7	53	11	15	5
1.68 to 2.0	2	7	9	23	3
2.0 to 3.0	0	7 } 14	35 } 81	31 } 70	38 } 92
3.0 to 4.0	0	0	29	16	30
4.0 to 5.0	0	0	8	0	21

Liver Disease Other Than Laënnec's Cirrhosis.—In Table III are shown the thymol turbidities of sixteen patients with other forms of liver disease than Laënnec's cirrhosis. The highest values were found in infectious hepatitis, but values above normal were also found in the sera from patients with hemolytic anemia with liver disease, carcinoma with liver metastases, and homologous serum jaundice. High values were also found in infectious mononucleosis, but this cannot be taken alone as evidence of the presence of liver disease in this condition.

TABLE III. THYMOL TURBIDITY IN LIVER DISEASE OTHER THAN LAËNNEC'S CIRRHOSIS

DISEASE	NUMBER OF PATIENTS	THYMOL TURBIDITY (C.C. $BaSO_4$ SUSPENSION)
Hemolytic anemia with liver disease	2	1.10, 2.30
Biliary cirrhosis	1	0.95
Familial nonhemolytic icterus	1	1.05
Hepatic vein obstruction	1	0.90, 1.00, 0.25
Carcinoma with liver metastases	4	0.90, 2.00, 1.75, 2.65
Infectious hepatitis	4	1.45 to 7.10
Infectious mononucleosis	2	1.50, 4.60
Homologous serum jaundice	1	1.30 to 4.85

Patients Without Evidence of Liver Disease.—The thymol turbidity was measured on the sera of twenty-seven hospitalized patients with illnesses not associated with liver disease. The results are shown in Table II where they may be compared with those obtained in Laënnec's cirrhosis. Only 14 per cent had turbidity values of 1.68 c.c. of barium sulfate or more.

COMMENT

The thymol turbidity described by Maclagan[1] has the advantage over most tests depending upon qualitative or quantitative changes in the serum proteins of being simple and rapid. In thirty-one normal individuals, Watson and associates[4] found values of from 1 to 4 Maclagan units and Shank and Hoagland[5] of from 0. to 4.7. The range of values in cirrhosis of the liver and infectious hepatitis is quite wide, but many are within or near the normal range. Thus, it is often difficult to decide whether a value is abnormal or not.

The modification of the method described herein makes it possible to determine more precisely the range of normal as well as the significance of abnormal values without the use of arbitrary units. Inspection of Table II shows that only 2 per cent of normal individuals had values above 1.68 c.c. of barium sulfate; however, 14 per cent of hospitalized patients without evidence of liver disease were above normal, and 30 per cent of the determinations in mild cirrhosis were within the normal range. Thus, a negative test is of no value in eliminating cirrhosis of the liver. However, a positive test equivalent to 4 c.c. of barium sulfate suspension or above is good evidence of liver disease, as none of the sera obtained in illnesses without liver disease were above this figure.

The cases of other forms of liver disease are too few for statistical analysis. However, Shank and Hoagland,[5] in infectious hepatitis, found eighty-two of

eighty-three patients with increased thymol turbidity, and Cohn and Lidman,[7] in fifteen soldiers with infectious mononucleosis, found a high incidence of positive reactions, as well as other evidence of liver disease.

SUMMARY

1. A modification of the Maclagan thymol turbidity reaction of serum is described, using the Evelyn photoelectric colorimeter.

2. For greater accuracy and convenience, the values for turbidity are expressed as cubic centimeters of the barium sulfate suspension used as standard. The approximate Maclagan units determined visually are presented for comparison.

3. The thymol turbidity of 105 apparently healthy young men varied from 0.16 to 2.19 c.c. of barium sulfate suspension. The upper limit of normal, using three times the standard deviation, was 1.68 cubic centimeters.

4. In cirrhosis of the liver, 81 per cent of the determinations were abnormal; in severe cirrhosis, 92 per cent. In a series of illnesses without evidence of liver disease, 14 per cent were above normal but none above 3 c.c. of barium sulfate suspension; whereas, 37 per cent of the determinations in cirrhosis were above this figure.

REFERENCES

1. Maclagan, N. F.: Thymol Turbidity Test: A New Indicator of Liver Dysfunction, Nature, London **154**: 670, 1944.
2. Maclagan, N. F.: The Thymol Turbidity Test as an Indicator of Liver Dysfunction, Brit. J. Exper. Path. **25**: 234, 1944.
3. Recant, L., Chargaff, E., and Hanger, F. M.: Comparison of the Cephalin-Cholesterol Flocculation With the Thymol Turbidity Test, Proc. Soc. Exper. Biol. & Med. **60**: 245, 1945.
4. Watson, C. J., Rappaport, E. M., Hawkinson, V., and Giebenhain, M.: A Comparison of the Results Obtained With the Hanger Cephalin-Cholesterol Flocculation Test and the Maclagan Thymol Turbidity Test in Patients With Liver Disease, J. Lab. & Clin. Med. **30**: 983, 1945.
5. Shank, R. E., and Hoagland, C. L.: A Modified Method for the Quantitative Determination of the Thymol Turbidity Reaction of the Serum, J. Biol. Chem. **162**: 133, 1946.
6. Ducci, H., and Watson, C. J.: The Quantitative Determination of the Serum Bilirubin With Special Reference to the Prompt-reacting and the Chloroform-soluble Types, J. Lab. & Clin. Med. **30**: 293, 1945.
7. Cohn, C., and Lidman, B.: Hepatitis Without Jaundice in Infectious Mononucleosis, J. Clin. Investigation **25**: 145, 1946.

LABORATORY METHODS

SEMIQUANTITATIVE ESTIMATION OF BILIRUBIN IN THE URINE BY MEANS OF THE BARIUM-STRIP MODIFICATION OF HARRISON'S TEST

C. J. Watson and Violet Hawkinson
Minneapolis, Minn.

THE importance of detecting bilirubin in the urine has recently been emphasized with respect to the diagnosis of infectious hepatitis, both in the preicteric stage of the disease and in those cases in which jaundice does not become manifest.[1-4] The barium-strip modification of the Harrison test for bilirubin in the urine, as described in a previous communication,[5] was sought and elaborated in order to provide a method which would be suitable for mass and serial usage, as in an epidemic of infectious jaundice, or in a chemical industry where injury of the liver was a possible hazard. Further experience with this technique has confirmed our belief in its simplicity and the rapidity with which it may be executed, also its specificity and sensitivity. The only other method which might be compared with it, on the basis of these attributes, is the methylene blue test, which, however, is not quite as sensitive nor as uniformly specific for bilirubin and in addition is more time consuming.

A description of the barium-strip modification of Harrison's test is given in the following:

Sheets of thick retentive filter paper* are thoroughly immersed in a saturated aqueous solution of barium chloride, after which they are dried either in a drying oven or at room temperature. The sheets are then cut into strips 3 or 4 inches by ½ inch in dimension. These strips may be prepared in any desired number, as they keep indefinitely.

To carry out the test, one of the barium-impregnated strips is simply inserted perpendicularly into the urine sample in question. It is held at the same level for from five to ten seconds, and at least ¼ inch of the lower end of the strip should be below the surface of the urine. During this period it is seen that the urine runs up the strip, but the pigments are adsorbed and collected just at the surface. The strip is removed and placed in the horizontal position on white absorbent paper such as ordinary filter paper or paper towel. At the point where the strip was at the surface of the urine, a yellow or brown line, or zone, is seen running transversely across the strip. This is usually present, although faintly, even with most normal urines. The Fouchet reagent, as used in the Harrison test, is dropped from a dropping bottle directly on this zone which was just at the surface of the urine. A green

From the Department of Medicine, University of Minnesota Hospital.
Received for publication, May 6, 1946.

*Schleicher and Schull, No. 470. Thus far, we have not had experience with any other type of paper which was satisfactory.

J. LAB. & CLIN. MED.
VOL. 31, NO. 8, AUGUST, 1946

BARIUM-STRIP MODIFICATION OF HARRISON'S TEST FOR BILIRUBIN IN THE URINE

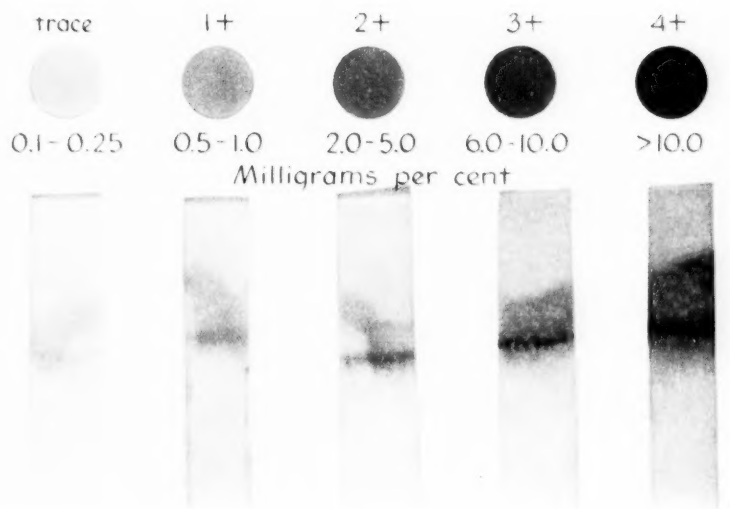

Fig. 1.

color denotes the presence of bilirubin. If just a trace is present, one may see only a very faint green line, while with larger amounts, an intense dark green is observed to spread up and down the strip over a broad area. The green color alone is specific for bilirubin. At times a purple or reddish color is seen, most likely due to dehydrogenation products of urobilinogen.

The foregoing procedure has been rendered semiquantitative by the use of a color chart of five standard intensities of green color, as shown in Fig. 1. These colors were painted* from the barium-strip Harrison tests of as many concentrations of bilirubin, each dissolved in pooled normal urine. The sample barium-strip tests, as shown in the lower part of Fig. 1, were carried out on pathologic urines containing varying amounts of bilirubin. They simply illustrate the means by which the comparisons with the standards are made.

This simple, semiquantitative method facilitates observation of the degree of bilirubinuria, whether increasing or decreasing, and thus aids in establishing the trend of the disease. Furthermore, it offers a more nearly standard means of reporting the degree of bilirubinuria which hitherto has been largely a matter of the personal equation.

SUMMARY

A standard color chart is reproduced by means of which the barium-strip modification of the Harrison test for bilirubin in the urine has been made semiquantitative in character.

REFERENCES

1. Neefe, J. R., Stokes, J., Jr., Reinhold, J. G., and Lukens, F. D. W.: Hepatitis Due to the Injection of Homologous Blood Products in Human Volunteers, J. Clin. Investigation **23**: 836, 1944.
2. Gellis, S. S., and Stokes, J., Jr.: The Methylene Blue Test in Infectious Hepatitis, J. A. M. A. **128**: 782, 1945.
3. Neefe, J. R., Stokes, J., Jr., and Gellis, S. S.: Homologous Serum Hepatitis and Infectious (Epidemic) Hepatitis, Am. J. M. Sc. **210**: 561, 1945.
4. Pollock, M. R.: Missed Cases of Infective Hepatitis, Brit. M. J. **2**: 598, 1945.
5. Hawkinson, V., Watson, C. J., and Turner, Roy H.: A Modification of Harrison's Test for Bilirubin in the Urine, J. A. M. A. **129**: 514, 1945.

*We are indebted to Miss Jean Hirsch, University Medical Art Shop, for this work.

APPLICABILITY OF THE BIURET REACTION TO THE DETERMINATION OF SERUM ALBUMIN BY METHANOL PRECIPITATION

HALVOR N. CHRISTENSEN, PH.D.
COOPERSTOWN, N. Y.

PILLEMER and Hutchinson[1] have shown that the precipitation of globulins by aqueous methanol under controlled conditions yields results for serum albumin comparable to those obtained by electrophoretic measurement. The protein partition obtained with sodium sulfate according to Howe[2] is known to yield falsely high values for albumin.[3, 4] In this laboratory the method of Pillemer and Hutchinson has been found convenient and useful. Further simplification has been obtained through the observation that albumins can be determined in the filtrates by the biuret reaction essentially according to Kingsley,[5] in spite of the presence of about 40 per cent methanol. This procedure avoids the difficulties inherent in the complete recovery of protein nitrogen by micro-Kjeldahl procedures.[6]

PROCEDURE

A 1 c.c. aliquot of the methanol-albumin supernatant (separated by spinning in a small centrifuge kept in the refrigerator) is transferred to a dry cuvette. In another cuvette is placed 0.1 c.c. of the original serum (for the total protein determination) and in a third cuvette (for the reference blank), 0.1 c.c. of water. To the latter two tubes is added 0.9 c.c. of acetate-methanol mixture (made by mixing 1 volume of acetate buffer, 7 volumes of aqueous methanol, both prepared according to Pillemer and Hutchinson, and 1 volume of water). To all cuvettes are added with mixing 4.3 c.c. of 3 N sodium hydroxide and 0.7 c.c. of 1 per cent copper sulfate. After just fifteen minutes the transmittances are determined in a photoelectric colorimeter or spectrophotometer. The absorption maximum is at a wave length of about 555 millimicrons.

The colorimeter is calibrated by measuring transmittances of several dilutions of a serum whose protein content has been determined by the Kjeldahl methods. The figures read from the curve relating log transmittance to per cent protein are halved for the albumin determination. With lactescent or highly icteric sera one may add 2 c.c. of ether as suggested by Kingsley. However, the correction of + 8 per cent used by Kingsley is omitted since dilution produced by dissolution of ether is approximately compensated by extraction of methanol (Table I).

With the spectrophotometer* at wave length 555 millimicrons, a linear relationship was obtained between log transmittance and concentration, the color being about 6 per cent more dense than that obtained in the absence of methanol.

From The Mary Imogene Bassett Hospital.
Received for publication, June 12, 1946.
*Model 11, Coleman Electric Co.

TABLE I. EFFECT OF ETHER ADDITION UPON TRANSMITTANCE OBTAINED WITH SERUM

SERUM	PER CENT TRANSMITTANCE	
	ETHER NOT ADDED	ETHER ADDED
1	31.3	31.3
2	36.3	35.9
3	30.8	29.9
4	26.5	26.0

TABLE II. COMPARISON OF ALBUMIN FOUND IN METHANOL FILTRATES BY BIURET REACTION AND BY KJELDAHL TECHNIQUE

SERUM	PER CENT SERUM ALBUMINS FOUND	
	BY KJELDAHL	BY BIURET REACTION
5	2.87	2.8
6	3.34	3.3
7	2.42	2.3
8	3.69	3.8

The presence of methanol apparently did not reduce the accuracy of the biuret method (Table II).

SUMMARY

The biuret method of Kingsley has been adapted to the measurement of serum albumin in filtrates obtained by methanol by the method of Pillemer and Hutchinson.

REFERENCES

1. Pillemer, L., and Hutchinson, M. C.: The Determination of the Albumin and Globulin Contents of Human Serum by Methanol Precipitation, J. Biol. Chem. **158**: 299, 1945.
2. Howe, P. E.: The Determination of Proteins in Blood—a Micro-Method, J. Biol. Chem. **49**: 109, 1921.
3. Butler, A. M., and Montgomery, H.: Solubility of Plasma Proteins, J. Biol. Chem. **99**: 173, 1933.
4. Majoor, C. L. H.: The Possibility of Detecting Individual Proteins in Blood Serum by Differentiation of Solubility Curves in Concentrated Sodium Sulfate Solutions, Yale J. Biol. & Med. **18**: 419, 1946.
5. Kingsley, G. R.: The Determination of Serum Total Protein, Albumin, and Globulin by the Biuret Reaction, J. Biol. Chem. **131**: 197, 1939.
6. Chibnall, A. C., Rees, M. W., and Williams, E. F.: The Total Nitrogen Content of Egg Albumin and Other Proteins, Biochem. J. **37**: 354, 1943.

OXYGEN CONSUMPTION AND DRUG ACTION: A METHOD FOR MEASUREMENT OF THE RESPIRATION OF AQUATIC ANIMALS

W. A. Robbie, Ph.D., and P. J. Leinfelder, M.D.
Iowa City, Iowa

MANY drugs affect tissue respiration, and in order to study the relationship between drug action and cellular metabolism it is frequently necessary to make measurements of the rate of oxygen consumption. With isolated cells or tissue slices the Warburg manometric technique is applicable; however, with larger intact organisms it is impractical to maintain oxygen equilibrium by shaking the immersion fluid, and some other method must be used. A modification of the constant-flow manometric respirometer, described previously for use with small mammals,[1] has been shown to be suitable for this purpose. The method is simple and determinations may be made at frequent intervals. This article describes the technique and presents illustrative results obtained with the frog and the quahog, *Venus mercenaria*, using hydrocyanic acid as the experimental drug.

Fig. 1 is a photograph of the equipment in operation. It consists essentially of three plastic chambers immersed in a constant-temperature water bath. Two of these are connected with a pump that continuously circulates air through them, and the third chamber is attached to a Warburg manometer and acts as a thermobarometer. The chambers are made of 4¾ inch lucite tubing with ⅛ inch walls, closed at the bottom with a plate of plastic. They fasten tightly to the ⅜ inch top plate by means of a simple inclined plane-locking device. This connection is sealed with a lanolin-petrolatum-mineral oil mixture each time the chambers are closed.

Air from the pump bubbles through the water in the first chamber, which contains the experimental animal, and then passes through a connecting glass tube to the second chamber. Carbon dioxide is removed from here by a tube of 5 per cent potassium hydroxide solution and an alkali-soaked gauze. The air is then led back to the pump through a thick-walled capillary tube and repeats the circuit. A lead from the top of the second chamber goes to a Warburg manometer, and any change in pressure within the system is thus observable. Since the carbon dioxide is absorbed and the temperature remains constant, the pressure changes (after correction for barometric changes recorded by the thermobarometer) are due to oxygen consumption by the animal in the water.

Air is circulated through the system by a rubber-tube pump which delivers about 250 c.c. a minute. A ⅜ by 22 inch piece of soft gum rubber tubing is coiled once inside a cylindric hole in a wooden block, with each end passing through the block and attaching to glass connecting tubes. The air is continuously driven through the rubber tubing by a free-moving wooden roller

From the Department of Ophthalmology, College of Medicine, State University of Iowa.
Received for publication, May 31, 1946.

which pushes the air ahead of it as it squeezes the rubber. This roller is driven by friction contact with another roller of about the same diameter, which is connected to the driving motor with a rubber coupling. The driving motor is one that is used for a Warburg manometer shaker and has a gear reduction which gives a speed of 120 revolutions per minute.

When the closed arm of the manometer is reset to the starting level before each reading, the system constitutes a constant volume type of respirometer, and the theory is similar to that of the direct method of Warburg.[2] If the volume and the temperature are kept constant, it is only necessary to multiply the thermobarometer-corrected manometer reading by a constant to determine

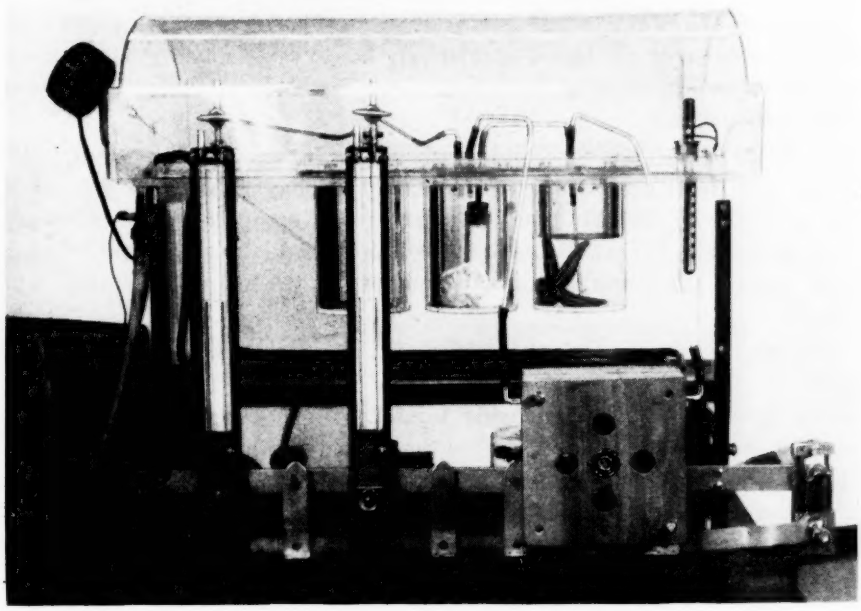

Fig. 1.—Photograph of closed-circuit manometric respirometer for measuring the oxygen consumption of aquatic organisms.

the amount of oxygen consumed, corrected to standard temperature and pressure conditions. This constant depends primarily upon the gas volume of the system, and the sensitivity may be varied over a wide range by changing the size of the chambers or the amount of fluid they contain. The total volume may be determined easily by measuring the amount of water the system will hold.

DETAILS OF TECHNIQUE

Oxygen equilibrium between the water and the air in the system is attained rapidly when the stream of flowing air is sufficient, and in most cases the lag in manometric response to a change in the respiration of the animal is insignificant. The water in the chambers should be allowed to come to the temperature of the thermoregulated bath before the initial readings are taken.

To maintain an equivalent vapor tension, the thermobarometer chamber should contain a small quantity of water.

Addition of oxygen to replace that which has been used by the animal is made by means of a rubber anesthesia bag filled with the gas and attached to the top opening of the manometer. The system is brought back to the original oxygen tension at any time by simply turning the stopcock of the manometer and allowing the gas to flow in.

Removing the carbon dioxide from sea water leads to an increase in alkalinity,[3] and since the potassium hydroxide in the second chamber combines with this gas, a change in pH may occur if marine animals are being studied. A buffer may be added,[4] but ordinarily this is unnecessary, since the carbon dioxide production of the respiring material is usually sufficient to maintain equilibrium. When the volume of the liquid is relatively large, the change in pH is insignificant for experiments of a few hours' duration, even if there is little carbon dioxide production.

Several connections are made with rubber tubing, and it is advisable to test the equipment occasionally for leaks. This may be done by setting it up without respiring material and comparing the readings with those of the thermobarometer. If the pump is well made and a good quality of gum rubber is used, the pump tubing will run for months before it requires replacement.

The apparatus is particularly well suited for observations of the effect of cyanide on oxygen consumption. This may be done by inserting a bubbler of calcium cyanide-calcium hydroxide mixture in the second chamber to add hydrogen cyanide gas to the air after the carbon dioxide has been removed. It has been shown that the hydrogen cyanide tension of such a mixture varies with the concentration of the calcium cyanide and with the temperature,[5] and it is thus possible, by use of the proper solution, to maintain a constant concentration of cyanide in the animal chamber and still remove the carbon dioxide

TABLE I. CALCIUM CYANIDE SOLUTIONS FOR MAINTAINING CYANIDE EQUILIBRIUM WITH WATER AT 20° C. (APPROXIMATELY 10 PER CENT CALCIUM HYDROXIDE SUSPENSION INCLUDED IN EACH ONE)*

CONCENTRATION OF HCN IN WATER (M.)	CALCIUM CYANIDE CONCENTRATION FOR EQUILIBRIUM (M.)
0.010	1.41
0.0046	1.23
0.0022	0.87
0.0010	0.56
0.00046	0.31
0.00022	0.16
0.00010	0.089
0.000046	0.048
0.000022	0.026
0.000010	0.014
0.0000046	0.0074

*If carbon dioxide-free air is bubbled through the calcium cyanide solutions listed in the second column, it may then be passed through the hydrocyanic acid solutions listed in the first column without changing their concentrations.

which is produced. In Table I is listed a series of calcium cyanide-calcium hydroxide mixtures with their respective hydrogen cyanide equilibrium levels for either fresh or sea water at 20° centigrade.

In the cyanide experiments, a measurement of the oxygen consumption under control conditions is made first; then the proper quantity of a hydrocyanic acid solution one hundred times the desired final concentration is added to the water in the animal chamber through one of the top tubes. At the same time a clamp on a Y tube leading from the second chamber is closed to make the carbon dioxide-free air bubble through a tube containing 30 c.c. of calcium cyanide-calcium hydroxide mixture and then through a small tube of boric acid solution (which removes any ammonia that may be liberated from strong cyanide solutions). In this way the gas which returns to the animal chamber is always in equilibrium with the fluid in it, and even though the experiment is continued for hours, the concentration of the liquid will not change.

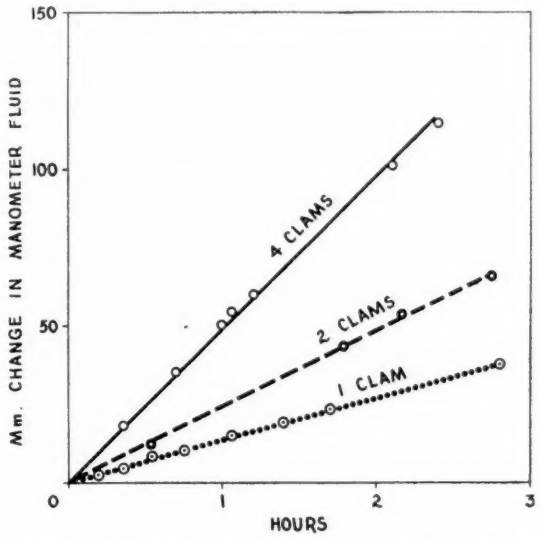

Fig. 2.—Respiration of the quahog, *Venus mercenaria*, as determined by the manometric respirometer. Ordinates indicate the change in the level of the manometer fluid at the times shown on the abscissas.

EXPERIMENTAL RESULTS

The apparatus has been tested in a variety of ways, and some of the experimental results are shown in Figs. 2 to 4. In Fig. 2 is illustrated the measurement of the oxygen consumption of the quahog or clam, *Venus mercenaria*. On the lowest curve is plotted the change in manometer reading with time when the chamber contained one animal in 1 liter of sea water. The middle curve shows the measurement when two animals were included, and the top curve that for four specimens.

In Fig. 3 are shown data from an experiment which demonstrates the effect of cyanide on the respiration of the quahog. Points along the upper curve were

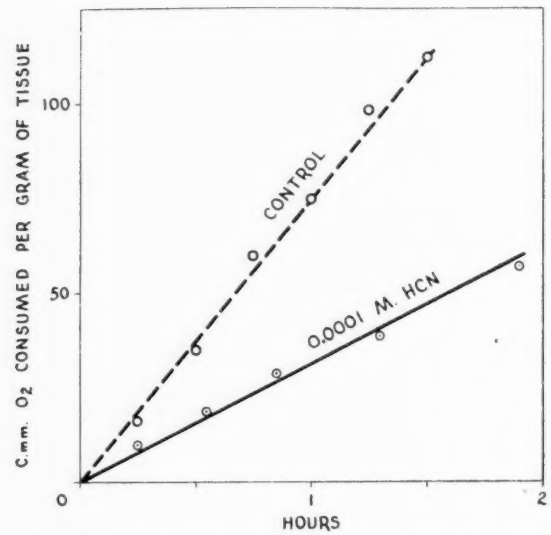

Fig. 3.—Oxygen consumption of an adult quahog during a control period and while it was immersed in 0.0001 M. HCN in sea water.

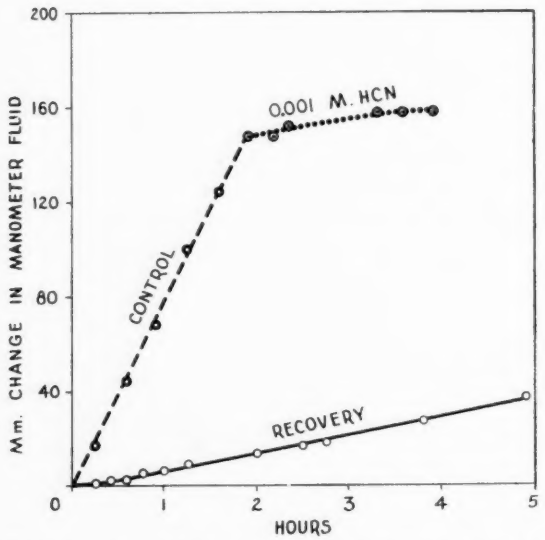

Fig. 4.—Manometric measurement of the oxygen consumption of a frog for control period during immersion in 0.001 M. HCN and for a recovery period after removal of the cyanide solution.

obtained during the control period. When enough 0.01 M. hydrocyanic acid was added to give a final concentration of 0.0001 M., the respiration fell off markedly as indicated by the lower curve.

In another experiment, a frog was allowed to sit on a platform half submerged in 1 liter of tap water. The curves in Fig. 4 show the course of the control respiration, the effect of adding cyanide (0 001 M. final concentration), and the course of the recovery after the water containing the cyanide was replaced with fresh water and the stream of hydrogen cyanide gas was shut off.

These experiments demonstrate the sensitivity of the method and illustrate the consistency of the results.

SUMMARY

An apparatus is described for making continuous measurements of the oxygen consumption of aquatic organisms. It is essentially a constant volume closed-circuit respirometer with a continuously flowing stream of air and manometric measurement of pressure changes. The sensitivity may be varied by adjustment of the volume of the system. It is useful in cyanide inhibition studies, for which purpose a table of calcium cyanide-calcium hydroxide mixtures that will maintain hydrogen cyanide equilibria is given. Illustrative experiments are reported.

The authors wish to thank Mr. Lee Allen for constructing the equipment.

REFERENCES

1. Robbie, W. A., and Leinfelder, P. J.: A Manometric Apparatus for Respiratory Studies of Small Animals, Science **101**: 48, 1945.
2. Dixon, Malcolm: Manometric Methods as Applied to the Measurement of Cell Respiration and Other Processes, New York, 1934, Cambridge University Press.
3. Whitaker, D. M.: On the Rate of Oxygen Consumption by Fertilized and Unfertilized Eggs. IV. Chaetopterus and Arbacia Punctulata, J. Gen. Physiol. **16**: 475, 1933.
4. Robbie, W. A.: The Quantitative Control of Cyanide in Manometric Experimentation, J. Cell. & Comp. Physiol., June, 1946.
5. Robbie, W. A., and Leinfelder, P. J.: Calcium Cyanide Solutions as Constant Sources of Hydrogen Cyanide Gas for Animal Experiments, J. Indust. Hyg. & Toxicol. **27**: 269, 1945.

THE METHYLENE BLUE TEST FOR BILIRUBINURIA: CLINICAL AND SPECTROPHOTOMETRIC OBSERVATIONS

GORDON D. STOKES, M.D.,* EARL E. GAMBILL, M.D.,† AND ARNOLD E. OSTERBERG, PH.D.‡
ROCHESTER, MINN.

FRANKE,[1] in 1931, described the methylene blue test for the detection of bilirubin in the urine. In 1940 Foord and Baisinger[2] made a comparative study of the Rosenbach, iodine, Müller, Huppert, Nakayama, Hunter, Naumann, Zins, Harrison, and methylene blue tests for bilirubinuria. They reported the methylene blue test to be as sensitive and as satisfactory as any of the nonconcentration methods.

The present study was prompted by the appearance in the literature in 1945 of two reports in which the methylene blue test was employed with usefulness. The first report is that of Myers,[3] who used the test among employees working with tetrachlorethane and thereby subject to toxic hepatitis from such exposure. We were impressed with the finding that in some cases the result of the test became positive before the appearance of icterus and in some cases even prior to elevation of bilirubin in the serum. Also impressive was the report that the result of the test tended to become negative prior to return of the serum bilirubin to a normal level. The second report is that of Gellis and Stokes.[4] The test was employed in seventy-seven cases of infectious hepatitis and in 1,000 cases in which normal persons served as control subjects. Of interest was the finding of a positive result in some of the cases of infectious hepatitis before the icterus index became elevated and the return to negativity as the icterus began to subside.

Figge[5] has called attention to the fact that no chemical reaction takes place in the methylene blue test, since the test can be duplicated by holding a blue glass filter in front of a test tube containing either yellow urine or a solution of bilirubin. A tube containing a solution of bilirubin was immersed in a dilute solution of methylene blue. Figge noted the same green transmission band for the two superimposed but unmixed solutions as occurred when the solutions were mixed. He stressed the nonspecificity of the test by showing that penicillin, riboflavin, porphyrin, hemoglobin, or picric acid, when added to urine or water, will give false positive results for bilirubin.

Watson and associates[6] likewise have indicated that the test is not specific for bilirubin, since yellow urines from normal persons and yellow substances such as potassium dichromate or ferric chloride will produce a green color when added to methylene blue. These authors also presented evidence to indicate that the test is due to a blend of color rather than to a chemical reaction.

Received for publication, April 5, 1946.
*Fellow in Medicine, Mayo Foundation.
†Division of Medicine, Mayo Clinic.
‡Division of Biochemistry, Mayo Clinic.

The following questions were suggested as we read the foregoing reports: Is the test specific for bilirubin, or will other substances produce a similar reaction? Is the green color of a positive result due simply to a blend of colors, or is it due to some type of chemical reaction? At what concentration of bilirubin in the urine and in the blood does the result of the test first become positive? Is there any correlation between the degree of hyperbilirubinemia and the positivity of the test? If the test is so delicate for bilirubin that it may show a positive reaction in urine before the serum bilirubin becomes elevated and may become negative while the patient is jaundiced and hyperbilirubinemia is still present, then might there be substances other than bilirubin which are responsible for the color reaction? Is the test of any value in estimating hepatic damage irrespective of the degree of hyperbilirubinemia?

MATERIAL AND PROCEDURES

It seemed of interest to observe the results of this test on urines from patients who had various types of disease affecting the liver and biliary tree sufficiently to produce jaundice or at least hyperbilirubinemia and particularly to study by means of the spectrophotometer the nature of the color reaction. For the test we have followed, in general, the procedure used by Gellis and Stokes.[4] A 0.2 per cent aqueous solution of methylene blue chloride was freshly prepared. A pipette delivering approximately 20 drops per cubic centimeter was used. To 5 c.c. of urine (usually, but not always, collected before breakfast) were added 20 c.c. of tap water to make 25 c.c. of a 1:5 dilution. To this solution methylene blue was added drop by drop from the pipette until the initial green color of the mixture became blue. The result of the test was considered positive if 5 drops or more of methylene blue were required to change the color from green to blue. In most instances a check test was run in which methylene blue was added to 5 c.c. of undiluted urine until the green color changed to blue. The changes of color, particularly in darkly colored urines, often were less distinct by this method than by the dilution method. The latter method, therefore, was selected as the one of choice.

The clinical material on which this study was based consisted of twenty-eight patients who had conditions involving primarily the liver and the common bile duct. In all cases there was hyperbilirubinemia. In twenty-two cases jaundice was present.

RESULTS

In Table I is summarized the data for the twenty-eight cases. It should be noted that Cases 1 and 2 represent jaundice due to malarial treatment for syphilis; Cases 3 to 11 inclusive, jaundice due to hepatitis or cirrhosis; Cases 12 to 19 inclusive, jaundice due to benign stricture of the common bile duct or obstruction of the duct by calculus; Cases 20 to 23 inclusive, jaundice due to carcinomatous obstruction of the common bile duct; Cases 24 to 27 inclusive, jaundice of uncertain cause; and Case 28, jaundice due to familial hemolytic anemia.

TABLE I. SUMMARY OF DATA

CASE	DIAGNOSIS	BILIRUBIN (MG. PER 100 C.C. OF SERUM) DIRECT	INDIRECT	DROPS METHYLENE BLUE TO 25 C.C. URINE DILUTED 1:5	REMARKS
1	Jaundice due to malarial treatment for syphilis	10.6	2.5	5	Ninth day of treatment
2	Jaundice due to malarial treatment for syphilis	2.2	1.2	2	Ninth day of treatment
3	Intrahepatic jaundice	10.6	1.0	6	
4	Residual hepatitis with jaundice	15.8	1.3	6	Painless icterus, four months, recurrent
5	Intrahepatic jaundice (chronic alcoholism)	38.6	7.8	16	Painless icterus, one week; dark urine, two months
6	Chronic hepatitis (residuum of infectious hepatitis)	25.1	1.8	14	
7	Chronic hepatitis (chronic alcoholism)	0.6	0.5	2	
8	Chronic alcoholism, cirrhosis of liver with ascites	1.6	1.3	4	
9	Chronic alcoholism, hepatomegaly	0	1.4	2	
10	Cirrhosis of liver with ascites	1.6	1.7	5	
11	Chronic hepatitis (chronic alcoholism)	19.6	2.9	20	
12	Cholecystitis and cholelithiasis, common duct stones, pancreatitis, hepatitis	6.2	0.8	5	
13	Subacute cholecystitis with stone, common duct stone, hepatitis	1.5	0.4	4	
14	Chronic cholecystitis with stones, common duct stone.	2.0		5	
15	Stricture of common duct, biliary cirrhosis	39.5	3.9	16	
16	Recurrent stricture of common duct	7.6	0.7	4	Tested three weeks postoperative
17	Recurrent stricture of common duct, biliary cirrhosis	4.0	0.7	4	
18	Stricture of common duct	15.3* 3.2†	2.7	8* 3†	
19	Stricture of common duct with external biliary fistula, chronic hepatitis	1.6	0.4	1	
20	Carcinoma of head of pancreas (three weeks after cholecystojejunostomy)	12.4	0.5	3	Bilirubin was 126 mg. per 100 c.c. of serum preoperatively
21	Carcinoma of head of pancreas with hepatic metastasis	54.6	13.4	16	
22	Carcinoma of ampulla of Vater	29.9	1.4	9	
23	Carcinoma of pancreas (eleven days after cholecystogastrostomy)	17.6	1.1	4	Bilirubin was 54.6 mg. per 100 c.c. of serum preoperatively
24	Hepatic metastasis, ascites, primary in pancreas (?)	47.6	3.6	25	
25	Painless jaundice 2.5 months, diffuse hepatitis (?)	54.6	1.8	15	
26	Postcholecystectomy syndrome (recurrent biliary colic with jaundice)	0	1.0	2	No cause found at operation
27	Intermittent painless jaundice one month (subsiding)	10.5	0.8	3	
28	Familial hemolytic jaundice	0	5.1	4	

*Preoperative.
†Postoperative.

In 1918 van den Bergh[7] formulated the idea that the kidneys are impermeable at all times to indirect bilirubin, regardless of the concentration, but are permeable to direct-reacting bilirubin in concentrations of 2 mg. per 100 c.c. of serum or higher. In 1921 Lepehne[8] and in 1925 McNee and Keefer[9] confirmed this viewpoint. In 1933 Bensley,[10] correlating the type and degree of hyperbilirubinemia with bilirubinuria, observed that with few exceptions bilirubinuria was present when the blood contained direct-reacting bilirubin in any concentration. With only a few exceptions bilirubinuria was not present when the blood contained only the indirect-reacting type of bilirubin regardless of the concentration. Lichtman[11] stated that the evidence from various sources indicates that the kidneys are impermeable at all times to indirect but are permeable to direct-reacting bilirubin in concentrations of 2 mg. per 100 c.c. of serum or more.

TABLE II. COMPARISON OF DEGREE OF BILIRUBINEMIA WITH POSITIVITY OF METHYLENE BLUE TEST

CASE	DIRECT-REACTING BILIRUBIN (MG. PER 100 C.C. OF SERUM)	NUMBER OF DROPS OF METHYLENE BLUE REQUIRED
Group I. Negative Results (Less Than 5 Drops Methylene Blue)		
9	0	2
26	0	2
28*	0	4
7	0.6	2
13	1.5	4
8	1.6	4
19	1.6	1
2	2.2	2
17	4.0	4
16	7.6	4
27	10.5	3
20	12.4	3
23	17.6	4
Group II. Positive Results, Grade 1 (5 Drops Methylene Blue)		
10	1.6	5
14	2.0	5
12	6.2	5
1	10.6	5
Group III. Positive Results, Grade 2 (6 to 10 Drops Methylene Blue)		
3	10.6	6
18	15.3	8
4	15.8	6
22	29.9	9
Group IV. Positive Results, Grade 3 (11 to 15 Drops Methylene Blue)		
6	25.1	14
25	54.6	15
Group V. Positive Results, Grade 4 (16 or More Drops Methylene Blue)		
11	19.6	20
5	38.6	16
15	39.5	16
24	47.6	25
21	54.6	16

*In Case 28, although the direct-reacting bilirubin was zero, the indirect was 5.1 mg. per 100 c.c. of serum.

In Table II the cases have been arranged into groups according to the degree of positivity of the result of the test and the concentration of bilirubin in the serum (direct-reacting type) for each in order to study the relationship of these two factors.

As may be noted in Table II, the correlation between the degree of hyperbilirubinemia and the degree of positivity of the methylene blue test is not perfect. The lack of perfect correlation is illustrated by a comparison of Case 22 and Case 11, in which the concentrations of bilirubin were 29.9 mg. and 19.6 mg. per 100 c.c. of serum, respectively. The urine in Case 22 required only 9 drops of methylene blue, while the urine in Case 11 required 20 drops to change the color from green to blue. Attention is called to Cases 16, 17, 20, 23, and 27 of Group I, in which the bilirubin ranged from 4 to 17.6 mg. per 100 c.c. of serum, and yet the methylene blue test gave a negative result (less than 5 drops of methylene blue). In Case 23 the methylene blue test was made postoperatively when the concentration of bilirubin was 17.6 as compared with a preoperative concentration of 54.6 mg. per 100 c.c. of serum. If bilirubin spills over into the urine at concentrations in the blood serum of 2 mg. per 100 c.c. or higher, and if the methylene blue test is as sensitive as reports[2-4] imply, then it is hard to understand why the test gave negative results in the five cases mentioned. It might be that the renal threshold for bilirubin becomes altered in some manner as a result of severe and prolonged hyperbilirubinemia. Studies in this respect would be interesting.

TABLE III. COMPARISON OF METHYLENE BLUE AND NITRIC ACID TESTS

CONCENTRATION OF BILIRUBIN (MG. PER 100 C.C. OF URINE)	COLOR AFTER ADDING 4 DROPS METHYLENE BLUE TO 5 C.C. OF MIXTURE	GMELIN'S TEST
0	Blue	−
0.6	Blue	−
1.2	Blue	−
1.7	Blue	+
1.8	Blue	+
1.9	Blue	+
2.0	Blue green	+
2.1	Blue green	+
2.3	Green	+
2.5	Green	+
3.3	Green	+
5.0	Green	+

An attempt was made to determine the amount of bilirubin in the urine necessary to effect a positive result with methylene blue. Some of the pooled serum of jaundiced patients used in one of the experiments reported herein was added to normal urine to produce varying concentrations of bilirubin. Then these mixtures were treated both with methylene blue and with nitric acid on filter paper (Gmelin's test). The results are shown in Table III. The slightest trace of color in the Gmelin test became discernible at 1.7 mg. per 100 c.c. of urine. The result of the methylene blue test became positive when the concentration of bilirubin in the mixture was 2.0 mg. or more per 100 c.c.

of urine. It must be remembered that this is an artificial bilirubinuria produced by direct- and indirect-reacting bilirubin, and hence the results may not be applicable to the urine of a jaundiced patient.

SPECTROTRANSMITTANCE STUDIES

The spectrotransmittance curves of urines giving positive results of the methylene blue test and other solutions used in the following experiments were determined by the use of a Coleman Junior Clinical Spectrophotometer, Model 6. With this instrument the percentage of light transmittance can be read at any wave length between 400 and 700 millimicrons. The data then are conveniently plotted on a graph with transmittance as the ordinate, expressed in percentage, and wave length as abscissa, expressed in millimicrons. Spectrotransmittance curves of urines giving positive results of methylene blue tests were plotted. Each showed a curve characteristic of bilirubin with the maximal absorption of light between 425 and 450 millimicrons. The results of these spectrophotometric studies are shown in Figs. 1 to 5.

In Fig. 1 curve *a* represents the spectrotransmittance of urine of a normal healthy man. Curve *b* shows the spectrotransmittance of 5 c.c. of urine from the same person, to which has been added 2 drops of methylene blue to produce a blue color. The addition of the blue dye causes an absorption which is maximal at 660 millimicrons.

In Fig. 2 curve *a* represents the spectrotransmittance of urine from a jaundiced patient, a man who had cirrhosis of the liver. The concentration of bilirubin on the day of this test was 25.1 mg. per 100 c.c. of serum direct, and 1.8 mg. per 100 c.c. indirect, by the van den Bergh reaction. The urine was dark brown and therefore had to be diluted before the methylene blue test could be read. Curve *b* is the spectrotransmittance after 1 drop of the dye has been added to 5 c.c. of undiluted urine, yielding a clear green color. Curve *c* is the spectrotransmittance of the solution obtained when 5 c.c. of the same urine were diluted with 20 c.c. of tap water and 14 drops of methylene blue were added to produce a blue color. Here is seen a wider band of absorption (from 600 to 675 millimicrons) than in curve *b* because of greater concentration of methylene blue.

In Fig. 3 curve *a* is the spectrotransmittance curve of a solution of sodium bilirubinate with maximal absorption between 425 and 450 millimicrons. It is thus seen that a solution of sodium bilirubinate produces essentially the same transmittance curve as that of urine of a jaundiced patient (Fig. 2, curve *a*). Curve *b* represents the spectrotransmittance of a solution of methylene blue in distilled water. There is a maximal absorption of 660 millimicrons. Curve *c* is the spectrotransmittance of a green solution obtained by adding 4 drops of methylene blue to 5 c.c. of the sodium bilirubinate solution. There are two absorption bands, one from 425 to 450 millimicrons and the other at 660 millimicrons, showing that the green color is merely a blend of the two original colors. That there is no new substance formed is evidenced by the lack of any additional absorption band.

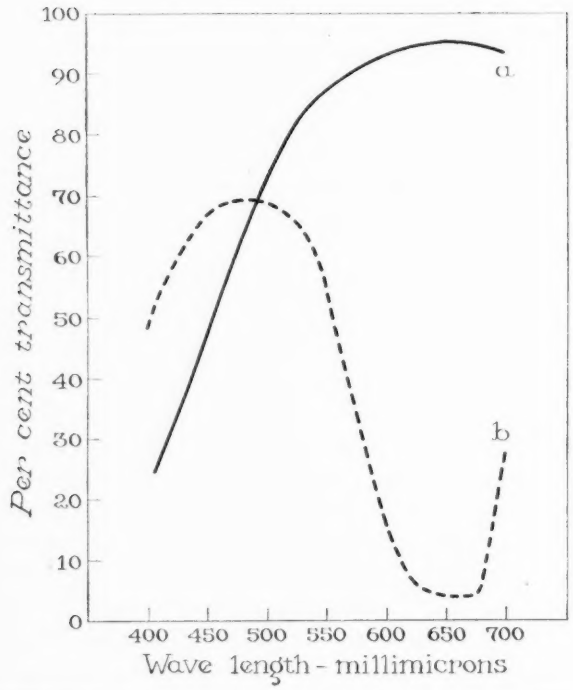

Fig. 1.—Spectrotransmittance (S-T) curves. Urine of a normal, healthy man: *a*, unmodified; *b*, with methylene blue added.

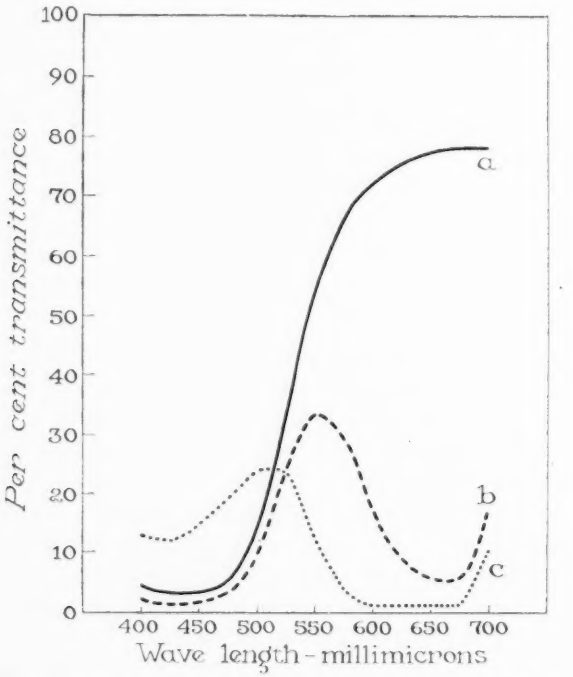

Fig. 2.—Spectrotransmittance (S-T) curves. Urine from a jaundiced patient: *a*, unmodified; *b*, with one drop of methylene blue added; *c*, with 14 drops of methylene blue added.

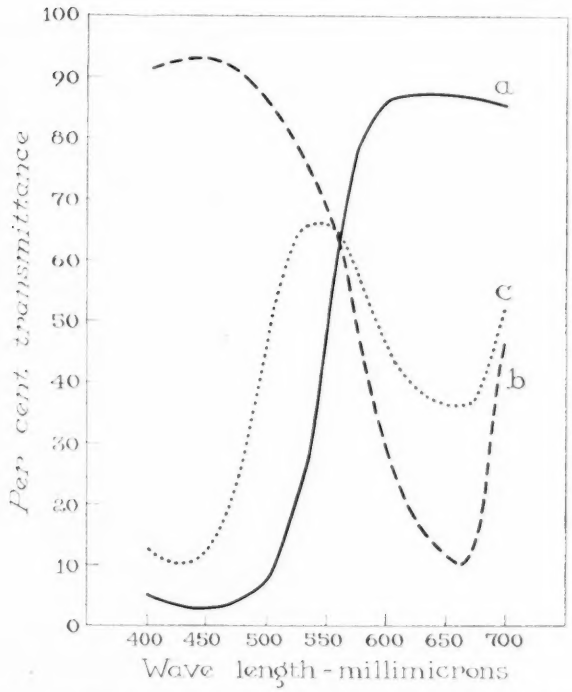

Fig. 3.—Spectrotransmittance (S-T) curves. *a*, A solution of sodium bilirubinate; *b*, a solution of methylene blue; *c*, a solution of sodium bilirubinate with methylene blue added.

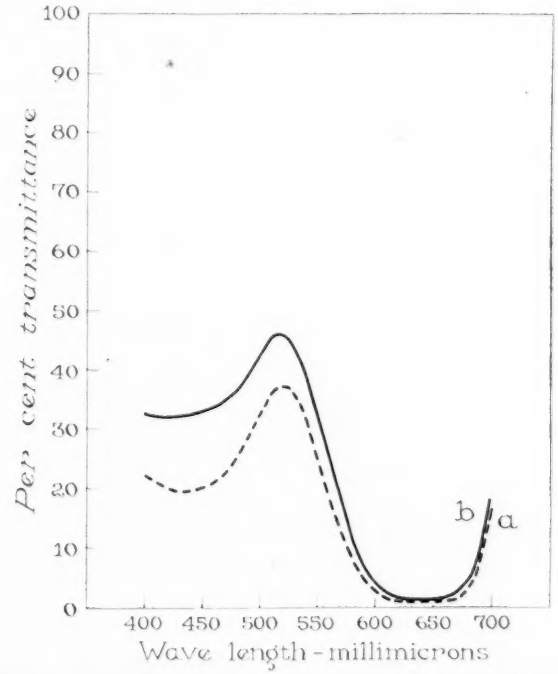

Fig. 4.—Spectrotransmittance (S-T) curves. *a*, Artificial bilirubinuria with methylene blue added; *b*, the same solution after further addition of methylene blue and further dilution with the same urine.

Fig. 4 represents the results of an experiment in which pooled serum containing 45.6 mg. per 100 c.c. direct- and 4.04 mg. per 100 c.c. indirect-reacting bilirubin was added to normal urine to give a concentration of 10 mg. of bilirubin per 100 c.c. of urine. Methylene blue was added drop by drop to produce a green—almost blue—color. The spectrotransmittance curve of this solution is represented by curve *a*. Then more methylene blue was added to produce a definite blue color. This solution was then diluted with more of the normal urine, and the color returned to green. The spectrotransmittance curve of the second green solution is represented by curve *b*. Because there has been no change in the curve, it may be reasoned that no new substance has been formed in the procedure.

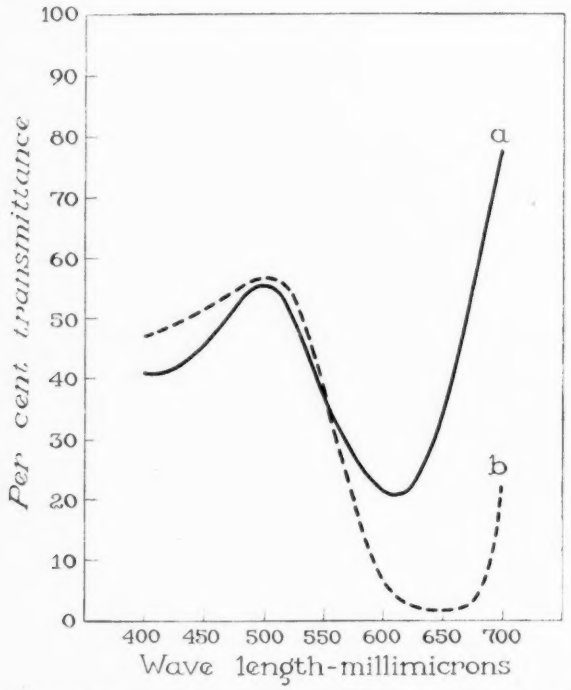

Fig. 5.—Spectrotransmittance (S-T) curves. *a*, Urine of jaundiced patients with Evans blue added; *b*, another specimen of the same urine with methylene blue added instead of Evans blue.

Urine for the determination, the results of which are shown in Fig. 5, was obtained from a patient who had jaundice due to stones in the common bile duct. A solution of Evans blue (T. 1824 dye) was made so that it had the same transmission of light as the methylene blue as measured by the spectrophotometer. Thus the solution of Evans blue and methylene blue were of the same intensity of color. Seven drops of Evans blue solution in 5 c.c. of urine were necessary to produce a blue color. The spectrotransmittance of the resulting blue solution is represented by curve *a*, with a maximal absorption at about 610 millimicrons. The urine was tested also with methylene blue, and 7 drops of the dye were required to produce a blue color in 5 c.c. of urine. The

spectrotransmittance curve of this solution is represented by curve *b*. This experiment tends to support the view that the color which results when methylene blue is added to urine which contains bilirubin is simply a blend of blue and yellow colors and can be duplicated by substituting a solution of Evans blue of the same intensity of color as the methylene blue.

SUMMARY

1. We did not observe perfect correlation between the concentration of bilirubin in the blood serum and the degree of positivity of the result of the methylene blue test.

2. The methylene blue test performed on urine with artificially produced bilirubinuria first became positive at a concentration of 2.0 mg. of bilirubin per 100 c.c. of urine.

3. Under the conditions of our experiments it would appear that the color which results when methylene blue is added to urine which contains bilirubin is due to a blend of blue and yellow colors rather than to some specific chemical reaction between methylene blue and bilirubin.

REFERENCES

1. Franke, Kurt: Methylenblau, ein einfaches sehr empfindliches Reagens zum Nachweis von Bilirubin, Med. Klin. **27**: 94, 1931.
2. Foord, A. G., and Baisinger, C. F.: Comparison of Tests for Bilirubin in the Urine, Am. J. Clin. Path. **10**: 238, 1940.
3. Myers, C. P.: Use of Methylene Blue in Testing for Bilirubin in the Urine, J. Indust. Hyg. & Toxicol. **27**: 52, 1945.
4. Gellis, S. S., and Stokes, J., Jr.: The Methylene Blue Test in Infectious (Epidemic) Hepatitis, J. A. M. A. **128**: 782, 1945.
5. Figge, F. H. J.: The Green Color of a Methylene Blue-Bilirubin Mixture, J. A. M. A. **128**: 613, 1945.
6. Watson, J., Meads, M., and Castle, W. B.: Tests for Urinary Bilirubin, J. A. M. A. **128**: 308, 1945.
7. van den Bergh, A. A. H.: Quoted by Lichtman.[11]
8. Lepehne, G. D.: Quoted by Lichtman.[11]
9. McNee, J. W., and Keefer, C. S.: Quoted by Lichtman.[11]
10. Bensley, E. H.: The Renal Threshold of Bilirubin, J. Biol. Chem. **103**: 71, 1933.
11. Lichtman, S. S.: Diseases of the Liver, Gallbladder and Bible Ducts, Philadelphia, 1942, Lea & Febiger, 906 pp.

A SIMPLE METHOD OF DETERMINING THE SPECIFIC GRAVITY OF SMALL SAMPLES OF URINE

LEE MONROE, M.D., AND JAMES HOPPER, JR., M.D.
SAN FRANCISCO, CALIF.

THE determination of the specific gravity of urine is a useful adjunct in judging renal function; however, it is often impossible to obtain a quantity sufficiently large for testing with an ordinary urinometer. Therefore, any method of determining specific gravity from a few drops of urine has possibilities of wide application, especially when it does so without requiring the use of a timing device or chart for interpretation.

The method described in this paper is a combination of two well-known techniques, that of Barbour and Hamilton[1] and that of Phillips and associates.[2] As in testing by the copper sulfate technique of Phillips, drops of urine are observed in insoluble media of known specific gravities, and, as in that method, there is no need to use a watch. Furthermore, the method herein presented can be used to measure the specific gravity of all watery solutions, as can that of Barbour and Hamilton. It does not require adjustment of mixtures while the test is in progress, as does the procedure of Kirkpatrick and Kling.[3]

For this determination a series of specific gravities covering the desired range may be prepared in 100 c.c. lots by mixing xylene and bromobenzene (XBB)* in the proper proportions. Solutions may be made either volumetrically according to the directions given in Table I or roughly by trial with a urinometer as a guide. The urine is introduced most easily under the surface of successive solutions in the graduated series by use of an ordinary dropper, the drops being displaced from the tip by an upward motion. The specific gravity of the urine lies between that of the mixture in which the drop rises and that in which it falls; or, if the drop neither rises nor falls in the bottle, the specific gravities of the urine and mixture are identical.

With ideal temperature control of numerous accurately prepared XBB mixtures, this method can be made equal in accuracy to the falling drop determination of Barbour and Hamilton.[1] Even using a roughly graded series and with ordinary fluctuations of temperature, this method is as accurate as regular determinations with a hydrometer. A series of mixtures covering the range from 1.005 to 1.029, inclusive, in rough fashion (that is, 1.005, 1.010, 1.015, 1.020, 1.025, 1.029) will give sufficient informatin to be of clinical value. For improved accuracy, slight changes in specific gravity due to evaporation of the mixtures can be minimized by corking the bottles tightly and can be corrected by the appropriate addition of xylene or bromobenzene. When XBB mixtures are used at temperatures widely variant from that at which they were prepared, correction should be made. Thus, the specific gravity should be changed

From the Division of Medicine, University of California Medical School.
Received for publication, April 30, 1916.
*Eastman Kodak, Xylene T275, Bromobenzene No. 43.

TABLE I

SPECIFIC GRAVITY	XYLENE (C.C.)	BROMOBENZENE (C.C.)
1.005	78.18	21.82
1.010	77.38	22.62
1.015	76.58	23.42
1.020	75.78	24.22
1.025	74.98	25.02
1.029	74.34	25.66

Thus, a 0.16 c.c. increase in the quantity of bromobenzene and an equal decrease in the amount of xylene will increase the specific gravity .001.

.0008[1] for each degree centigrade deviation in observed temperature from the calibration temperature (that is, if the XBB mixtures were originally prepared at 22° C. and the temperature at the time the test is made is 17° C., the specific gravity in each of the bottles should be corrected by adding .004).

After repeated use the mixtures may become cloudy so that observation of the progress of the drops is difficult. This is due to the accumulation of urinary sediment plus the excess of urine in the bottles. The mixtures may be easily cleaned by passing each through an average-grade filter paper, and the urine may be largely removed by decanting or by using a small separatory funnel.

This method, while useful in lieu of a urinometer in all determinations of the specific gravity of urine, has several specialized applications. The urologist, when called upon to test separately and comparatively a patient's kidneys, will find this a most sensitive technique to use in addition to the relatively crude phenolsulfonphthalein test; for with but a few drops of urine obtained from each ureteral catheter, he can measure accurately the tubular concentrating power of an individual kidney. Thus, a differential dehydration test for comparison of renal function is made possible. The pediatrician will find the method of value for determining the specific gravity of urine of babies and children whose specimens are scanty. Studies of renal function in small animals also will be facilitated, as the specific gravity of urine produced by a single animal may be measured and reliance on pooled specimens eliminated.

SUMMARY

A simple method for determining the specific gravity of small samples of any aqueous solution is presented for use in clinical and laboratory work.

REFERENCES

1. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, J. Biol. Chem. **69**: 625, 1926.
2. Phillips, R. A., Van Slyke, D. D., Dole, V. P., Emerson, K., Jr., Hamilton, P. B., and Archibald, R. M.: Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma, New York, 1945, Josiah Macy, Jr., Foundation.
3. Kirkpatrick, J., and Kling, D. H.: A Simplified Method for Determination of the Specific Gravity of a Few Drops of Urine, J. A. M. A. **87**: 487, 1926.

BOOK REVIEWS

Preventive Medicine and Public Health. *Wilson G. Smillie*, A.B., M.D., D.P.H., Sc.D. (hon.), New York, The Macmillan Company, New York, 1946. Price $6.00. 607 pages.

There can be little doubt that a great need exists for a textbook of preventive medicine and public health for the use of medical students and those whose job it is to teach medical students. There may be some who will claim that this book does not entirely meet that need, but practically everyone must admit that this is the best effort made to date to present the newer concepts of these subjecs.

The author states that this book was planned to introduce medical students to a "point of view"; that it was prepared for those who will eventually practice clinical medicine. The major thesis is that the physician in practice "has the obligation to his patients and to his community to prevent illness and to promote family and community health."

He subscribes to the present view that a distinction should be made between the terms "preventive medicine" and "public health," not in purpose or results achieved but in allocation of responsibility and in the organization of instruction of medical students. "Preventive medicine encompasses those activities that are the direct responsibility of the individual in the prevention of disease and in the protection and promotion of health of himself and his family." The responsibility for the execution of these activities falls upon the practitioner of medicine, who should be the family health adviser.

"Public health, on the other hand, encompasses those activities that are undertaken for the prevention of disease and the promotion of health which are primarily a community responsibility. These activities may be carried on by an official department of public health, by voluntary health promotion agencies, by departments of education, by state or county medical societies, or by other community agencies."

The point of view which Dr. Smillie wishes to present is that medicine is a "social science." In addition to this, the purposes of the book are threefold: (1) to present information concerning the responsibilities of the community in the protection and promotion of the health of its people; (2) to indicate to medical students methods for the incorporation of preventive medicine in the practice of clinical medicine; (3) to demonstrate the important functions of the doctor in health protection and promotion in the area in which he is engaged in practice.

He proposes to fulfill these purposes in his presentation without including much that is traditionally taught by departments of public health and by giving due consideration to the principles rather than the practices of public health. This book, therefore, has been reviewed with attention primarily focused upon these three purposes, chapter by chapter.

The text is in six sections, including an introduction, a discussion of environmental sanitation, communicable disease control, child hygiene, adult health protection and promotion, and, finally, a brief discussion of public health administration. The introduction is concerned with a definition, a discussion of population trends, and a chapter on vital statistics. Although the author discusses ageing of the population and changes in the birth and death rates, he does not emphasize sufficiently the effect of these changes upon medical practice. It is this reviewer's opinion that the discussion of vital statistics is weakened by the inclusion of detailed instruction in biometrics. Many will agree that quantitative methods in medicine should be taught to medical students at some phase of their medical education, but the question arises in this instance as to the wisdom of the discussion of ratios, variation, probabilities, etc., at this particular place in the text. It is well presented, however, and the discussion on common errors in the statistical interpretation of medical data is excellent.

The following eighty-eight pages on environmental sanitation represent a good, readable, brief discussion on sanitary control of the environment as a part of the community-wide public health program. This section should be read by all medical students, and indeed by their instructors as well.

The section on the control of communicable disease is disappointing in that the opportunity to fulfill the original purposes set out in the introduction was not used to advantage. In this section more than thirty diseases are briefly discussed, with particular emphasis on their control. It is readily understandable why the author did not go into detail about each of these diseases. It is just as readily understandable why he did not wish to devote the discussion to preventive aspects alone, since they depend upon and cannot be divorced from the clinical. It would seem preferable, however, to have presented a discussion of general principles, epidemiology, etc., *in terms of the original purposes,* with the objective of giving a point of view for application to the control of many of the communicable diseases.

In presenting the material on child hygiene the responsibilities of the community are reviewed, the functions of the doctor in health protection are emphasized, and the place of preventive medicine is well established. This section shows what should be accomplished on an individual and community basis with regard to child health.

The next section, on adult health protection and promotion, suffers from brevity, but the reader is referred to a more complete text on these subjects. The community aspects of mental hygiene, malnutrition, cancer, peptic ulcer, appendicitis, hypertension, obesity, diabetes, arthritis, allergic conditions, alcoholism, and industrial hygiene are only briefly mentioned, not enough information being given to appreciably assist students or clinical instructors in developing an understanding of these functions as they relate to the practice of medicine. The discussion of industrial hygiene is good.

The book terminates with a series of chapters devoted to public health practices in order to show how the various activities that have been previously mentioned are correlated into an over-all administrative plan.

In the preface the author emphasizes that this textbook is intended simply as a guide to further reading and investigation, but unfortunately references are relatively few and at times confusingly placed, occasionally at the end of a chapter and again at the end of a section.

The major defect in the work seems to be related to an attempt to cover too many topics in the field of medicine. This has led to all too brief discussions of these conditions and prevents the use of this book as it now stands as a reference book. It is good reading and has a wealth of important information, but it seems that the point of view could well have been created and the purposes fulfilled had fewer conditions been considered in more detail. This book is recommended as the best existing text on this subject now available.

E. GURNEY CLARK, M.D.

Journal of Gerontology. Edited by *Robert A. Moore,* M.D. Published by Charles C Thomas, Springfield, Ill. Subscription $6.00 per year.

The Journal of Gerontology, the first issue of which appeared in January, 1946, serves as a mouthpiece for The Gerontological Society, Inc., and is published under the supervision of this society. The society in turn is an outgrowth from the American Division of the International Club for Research on Ageing. This club was formed by a former pupil of Eli Metschnikoff, Professor V. Korenchevsky, who now carries on his research on ageing in the Department of Zoology and Comparative Anatomy of Oxford University, England.

The Journal of Gerontology, under the editorship of Professor Robert A. Moore, of the Medical School of Washington University, makes its appearance in two forms. First, as the official organ of the Gerontological Society, and acting in this capacity it has as its function to carry scientific articles and papers of a sociological and literary order primarily concerned with the ageing process in the lower animals, man and plants. Second, this Journal simultaneously appears as a nontechnical supplement. In this supplement the contributions

which are carried by the official Journal are not only abstracted but so restated without any factual change that the lay public can read and digest this basic scientific, economic, and sociological material. It is the hope of the Gerontological Society, as well as the editor of this Journal, that they may reach an ever expanding and varied order of reader interested in the general theme of ageing.

Volume 1, Number 1, January, 1946, of *The Journal of Gerontology* carries such varied contributions as the "Increase in Mortality as a Manifestation of Ageing" by Henry A. Simms, of the Department of Pathology of Columbia University Medical School; "Ageing in Nutritionally Deficient Persons" by Tom D. Spies and Harvey S. Collins, of the Nutrition Clinic, Hillman Hospital, Birmingham, Alabama; "Bugeting for Social Security" by W. R. Williams, Actuarial Consultant, Federal Security Agency; and "Shakespeare's Attitude Towards Old Age" by John W. Draper, Department of English, University of West Virginia.

The primary object of *The Journal of Gerontology* is to publish scientific papers, the products of research, which have as their objective an understanding of those changes of a chemical and physical order acquired by tissues as they advance from the commencement of the life of the organism through successive age segments towards senility and senile death. With such information this Journal would also have the high hope "to add life to years, not just years to life."

WM. DEB. MACNIDER.